Effects of rice straw burning products on guinea pig lungs

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This study aims to investigate rice open field burning and their deleterious effects which has become an obvious problem in Egypt, which apparently affects the Egyptians’ lungs and increased hospital admission for pulmonary complaints. Therefore, this study was designed to investigate this phenomenon by using guinea pigs which were subjected to rice straws burning products (RSBP). The effects of RSBP on differential leukocytic count in lung lavages, histopathological examination, malondialdehyde (MDA) content in lung tissues (marker of lipid peroxidation) and nitric oxide (NO) content in lung tissues were studied. Results of this study demonstrated that RSBP induced pulmonary emphysematous lesions are progressive with subsequent smoke exposures together with the sensitization of the lung in the present model. Changes in the count of macrophages, neutrophils and eosinophils in the bronchoalveolar lavage (BAL) of guinea pigs lungs compared to normal lavages. RSBP exposure has a potent potential capacity for being a source of reactive oxygen species and possibly oxidizing species which can lead to decrease nitric oxide content in lung tissue.

Key words: Rice, straws, smoke, guinea pigs, total leukocytic count, neutrophil and eosinophils count, macrophages count, nitric oxide content, malondialdehyde content, lung, histopathological examination.

INTRODUCTION

Over the last few years, Egyptian farmers in the middle delta tend to burn the rice straw in the rice field as an easy and cheap method for its disposal. At the same time, farmers burn the rice straws to kill insects and provide minerals to the soil (Estrellan et al., 2010). Agricultural field burning activities are linked to elevated air pollution levels in Asia, for example, Taiwan (Yang et al., 2006), Thailand (Tipayarom et al., 2007), USA (Jiminez et al., 2007) and Europe (Viana et al., 2008). Clinicians working in agricultural areas in delta counties are aware of an abrupt increase of patients suffering from asthmatic attacks after the harvesting is completed, and the remaining paddy straws have dried up. The patients themselves are aware that rice smokes aggravate their airway symptoms after the harvesting season (from September to November) every year. This can result in adverse health effects (Regalado et al., 2006; Yang et al., 2007; Ryu et al., 2007).

In recent years, it has been observed that open burning of crop residues also contributes to emissions of harmful
air pollutants, which can cause severe impacts on human health, including polycyclic aromatic hydrocarbons (PAHs) (Korenaga et al., 2001), as well as polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), referred to as dioxins (Gullett and Touati, 2003; Lin et al., 2007). These air pollutants have significant toxicological properties and are notably potential carcinogens. Air pollution not only affects human health and the environment, but also indirectly the economy of a country. Air pollutants and emission factors are considered for rice straw open burning. Open field burning is an uncontrolled combustion process during which species such as CO2, nitrous oxide (N2O), CH4, CO, non-methane hydrocarbons (NMHC), NOx, SO2, particulate matter (PM) and few others are being emitted as mentioned in Gadde et al. (2009) study. Among these, the greenhouse gases (GHGs) of importance are N2O and CH4 which contribute to global warming and climate change. CO2 emitted from biomass burning is considered to have a neutral effect due to its photosynthetic uptake during plant growth. Particulate matter (PM), because of their impacts on human health and the environment, can be further categorized as PM less than 2.5 micron (PM2.5) and PM less than10 micron (PM10). PAHs and PCDD/F are also of importance due to their toxicity and carcinogenic nature. Emission factors specific to air pollutant species emitted from open field burning of agricultural residues are presented in Gadde et al. (2009) study. This emission factors (EFs), which is the fraction of the mass combusted during the course of a fire, were collected from the literature and are mostly specific to rice straw burning. A larger number of people can be subject to rice straws burning products (RSBP) effects in the event that the dominant winds are directed towards more densely populated areas since many urban areas are typically located next to rice cultivated areas. Therefore, although rice cultivation has assumed great importance for the national economy, especially because of the growing demand for food in the current decade, it presents a high potential for environmental impact. This burning practice is likely to remain an important issue until effective measures are taken to eliminate this practice. The rice smoke is an important PAH emission source. The rice straw burning primarily contributes fine particulate PAHs. During the rice straw burning periods, PAH size distribution shows that 70.9% of PAH mass is distributed in the sizes smaller than 2.5 mm, which can easily penetrate the pulmonary alveoli and damage the health of humans (Hsi-Hsien et al., 2006; Chia-Hsiang et al., 2009). Particles from wood smoke have been associated with various indicators of inflammation, including pulmonary influx of inflammatory cells such as neutrophils, lymphocytes and alveolar macrophages (Larsson et al., 2007). An increase of neutrophils in the airway lumen has been documented by bronchoalveolar lavage (BAL) fluid and sputum analysis in smokers with mild to moderate chronic airway obstruction (Bohadana et al., 2004). The guinea pig cigarette smoke (CS) models have contributed to the understanding of histological and physiological aspects of smoke-associated lung disease. Guinea pig models are considered to be adequate for further investigations of chronic obstructive pulmonary disease (COPD) because of the anatomical and pathophysiological similarities to human COPD. The guinea pig develops morphologic and physiologic alterations after exposure to cigarette smoke at roughly the same concentrations as humans. In contrast, rats and mice require respectively greater and shorter exposure concentrations before developing disease than humans do (Kubo et al., 2005; Ricciardolo et al., 2008).

In this study, we used guinea pig model for exposure to RSBP, and sensitization with egg albumin for simulation of hypersensitivity reaction, in order to investigate various histopathological changes and bronchoalveolar lavages to see what possible cells are responsible for lung damage. The suspected effects of the RSBP on the content of malondialdehyde (MDA) in lung tissues as a marker of lipid peroxidation, and oxidative stress have also been investigated. Nitric oxide (NO) content in lung tissues was also studied.

MATERIALS AND METHODS

Normal healthy male guinea pigs (Egyptian breeding) weighing 400 to 500 g were used. The animals were kept in cages, in a standard animal laboratory room. They had free access to water and food at room temperature. At the moment of experiment, guinea pigs were all of the same age, and had approximately the same body weight in order to minimize biological variations. Rice straws were brought from nearby country area in Gharbiya County, Tanta, Egypt. The burning rate was adjusted to burn about 10 g of rice straws in about 20 min on an electric heater (Gallenkamp Magnetic Stirrer Hotplate 400, UK). It was adjusted at the middle heat which was about 200°C. The burnings were continued for 4 h per day in a burning chamber. The animal exposure was performed by direct inhalation of the smoke for 4 h. Smokes comes out from burning chamber by inlet pipe to exposure chamber made of wood of the following diameters 90×50×45 Cm with inlet opening for air atmosphere to enter. The area of the pipe of the inlet is about (1 cm²) (Six animals per each exposure chamber).

Egg albumin suspension (EA)

A suspension of egg albumin (Sigma Chemical Co. Ltd., Egypt) was prepared in normal saline to give a final concentration of 100 mg/ml and 10 mg/ml for sensitization of guinea pigs.

Induction of sensitization

Guinea pigs sensitization was induced according to a previously described procedure (Mcaig, 1987). The animals were injected subcutaneously and intraperitoneally I.P. with two equal doses of egg albumin and 1 ml of suspension containing 100 mg egg albumin (EA) on day one, and a further 10 mg I.P. on day eight. At
day 14, sensitized animals were exposed to an aerosol of 4% EA for 18±1 days, 4 min daily. The aerosol was administered in a closed chamber, dimensions 30x20x20 cm. Control group was treated identically, except that 0.9% saline vehicle alone was used. The animals were ready for exposure to the RSBP.

The experimental design

The animals were divided into six groups. Six guinea pigs were used in each group:

A. Control group: Non-exposed normal guinea pig.
B. Egg albumin sensitization group: Guinea pigs with sensitized lungs.
C. Acute RSBP exposed group: Normal guinea pigs exposed to RSBP for two weeks.
D. Subchronic RSBP exposed group: Normal guinea pigs exposed to RSBP for four weeks.
E. Sensitized acute RSBP exposed group: Sensitized guinea pig exposed to RSBP for two weeks directly after sensitization period.
F. Sensitized Subchronic RSBP exposed group: Sensitized guinea pig exposed to RSBP for four weeks directly after sensitization period.

Inflammatory cell analysis in bronchoalveolar lavage (BAL)

Guinea pigs were anaesthetized with pentobarbital sodium (60 mg/kg i.p.). The thorax was opened and the aorta and inferior vena cava were cut. The trachea was then cannulated and 10 ml of phosphate buffered saline at 37°C was gently introduced into the lung and then gently withdrawn. Three further washes with 10 ml of the saline were carried out. The combined lavage fluid was kept in an ice bath. The collected BAL was centrifuged at 1,500 rpm for 10 min, (GIBCO, Grand Island, NY). Total cell counts were determined by using (Burkerhemocytometer, NY). Differential leukocyte counts were then performed on cytospin slides. A minimum of 300 cells were identified and differentiated as mononuclear cells, neutrophils or eosinophils using the standard morphological criteria (Kubo et al., 2005). Points falling on a specific cell type must be counted, and then divided by the total number of points falling on tissue area in each microscopic field, as previously described (Gundersen et al., 1988)

Determination of lung lipid peroxides contents measured as malondialdehyde (MDA)

MDA lung tissue content was assayed as an indirect indicator of in situ lipid peroxidation (Yoshika et al., 1979). Briefly, lung tissues were homogenized in 10 volumes ice-cold 1.15% (w/v) potassium chloride solution using polytron homogenizer (PT 3100). To 0.5 ml of homogenate, 3 ml of 0.5% (w/v) trichloroacetic acid and 1 ml of 0.6% (w/v) thiobarbituric acid were added; the entresolution was then mixed and heated for 45 min in a boiling water bath. After cooling, 4 ml n-butanol was added and the sample vigorously shaken. The n-butanol layer was separated by centrifugation at 3000 rpm for 15 min. The absorbance of the pink colored product was measured at 535 nm against blank containing water instead of the sample, using double-beam spectrophotometer (Shimadzu UV-PC 1601, Japan).

Determination of lung nitric oxide contents

The lung nitric oxide content was determined by measuring its stable metabolites nitrite and nitrate (Miranda et al., 2001) briefly, lung tissues (= 0.25g/guinea pig) were homogenized in 10 volumes of ice cold saline (0.9% NaCl) using polytron homogenizer (PT3100). 1 ml absolute ethanol was added to 0.5 ml of the homogenate to precipitate proteins. Samples were centrifuged at 3000 rpm for 10 min. Addition of 0.5 ml saturated solution of vanadium (III) chloride (8 mgVCl₃/ml) to 0.5 ml of the clear supernatant was rapidly done, followed by addition of 0.5 ml freshly prepared gries reagent. The mixture was vortexed, and incubated at 37°C for 30 min in a water bath. The absorbance of samples was measured at 540 nm using double-beam spectrophotometer (Shimadzu UV-PC 1601, Japan).

Histopathological examination of lung sections

At the end of the rice smoke exposure, the lungs were immediately removed, washed with saline, and prepared for histopathological examination. The lungs was immediately fixed in 10% buffered formalin solution (pH 7.4) for 24 h, and then routinely processed in ascending grades of alcohol, then xylene. The tissues were then embedded in paraffin wax, serially-sectioned to ≈ 4 µm thickness, and stained with Hematoxylin and Eosin (H&E; Sigma). Ultimately, each stained tissue section was examined using a light microscope (Olympus BX 51, Olympus America, Melville, NY) and photographed with a digital camera (Olympus DP11) connected to the microscope.

Statistical analysis

The results represented as the mean ± SD of cell count change. The collected data were organized, tabulated and statistically analyzed using SPSS software (Statistical Package for the Social Sciences, version 16, SPSS Inc. Chicago, IL, USA). For comparison between more than two means of parametric data, F value of ANOVA test was calculated, where scheffe test was performed to compare between each two means if F value was significant. Significance was adopted at p<0.05 for interpretation of results of tests of significance (Dawson and Trapp, 2001) (Tables 1 and 2, Figures 15 to 18)

RESULTS AND DISCUSSION

The resulting hazards of smoke exposure arise from burning of rice straws and its deleterious effects on the lungs of exposed persons in Egypt. Fine particles, one of the major pollutants emitted from the field burning of rice straws, are of major concern due to their harmful effects on human health (Pope et al., 2009). Crop rice burning is a serious environmental health hazard, and children are more sensitive to air pollution, as RSBP poses some unrecoverable influence on their pulmonary function test (PFT) (Awasthi et al., 2010), early small airway obstruction (Ravinder et al., 2012). PAHs compounds have also been reported to induce a release of pro-inflammatory mediators, supporting a role for PAHs in the pro-inflammatory response (Lecureur et al., 2005). Substituted PAHs, such as nitro- and oxy-PAHs, have been suggested to affect biological systems by induction of oxidative stress, mitochondrial damage, necrosis and apoptosis (Xia et al., 2004; Kubatova et al., 2006; Landvik et al., 2007).
### Table 1. Mean values of blood cells in lung lavage of the study groups of guinea pigs (exposed and not exposed to rice straw burning products (RSBPs) (n=36).

<table>
<thead>
<tr>
<th>Findings of lung lavage</th>
<th>Unexposed to RSBPs (n=12)</th>
<th>The study guinea pigs (n=36)</th>
<th>Exposed to RSBPs (n=24)</th>
<th>F-value (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group (A) Control (n=6)</td>
<td>Group (B) Sensitized (n=6)</td>
<td>Group (C) Normal acute exposed (n=6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group (D) Normal subchronic exposed (n=6)</td>
<td>Group (E) Sensitized acute exposed (n=6)</td>
<td>Group (F) Sensitized subchronic exposed (n=6)</td>
<td></td>
</tr>
<tr>
<td>Total white blood cells (TWBC) (cell/μm²)</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Macrophages (cell/μm²)</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Eosinophils (cell/μm²)</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Basophils (cell/μm²)</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Median</td>
<td>Range</td>
</tr>
</tbody>
</table>

*Significant (P<0.05)
Table 2. Mean values of malondialdehyde (MDA) and nitric oxide content (NO) of the study groups of guinea pigs (exposed and not exposed to rice straw burning products (RSBPs) (n=36).

<table>
<thead>
<tr>
<th>Oxidative stress indicators</th>
<th>Unexposed to RSBPs (n=12)</th>
<th>Exposed to RSBPs (n=24)</th>
<th>The study guinea pigs (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group (A) Control (n=6)</td>
<td>Group (B) Sensitized (n=6)</td>
<td>Group (C) Normal acute exposed (n=6)</td>
</tr>
<tr>
<td>MDA (μM/g tissue)</td>
<td>Range</td>
<td>43-59</td>
<td>43-59</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>50.50±6.06</td>
<td>53.17±5.68</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>50.50</td>
<td>56.00</td>
</tr>
<tr>
<td>Scheffe test (P)</td>
<td>GA vs GD, P=0.0001*, GA vs GE, P=0.0001*, GA vs GF, P=0.0001*</td>
<td>GB vs GD, P=0.0001*, GB vs GE, P=0.0001*, GB vs GF, P=0.0001*</td>
<td>GC vs GD, P=0.0001*, GC vs GE, P=0.0001*, GC vs GF, P=0.0001*</td>
</tr>
</tbody>
</table>

NO (μM/g tissue)

| Mean±SD | 60.67±6.38 | 46.83±8.11 | 27.00±5.62 | 30.33±5.99 | 30.67±4.93 | 31.17±5.98 | (0.0001*) |
| Median | 61.50 | 43.00 | 26.00 | 29.50 | 30.00 | 31.50 | - |
| Scheffe test (P)            | GA vs GB, P=0.028*, GA vs GC, P=0.0001*, GA vs GD, P=0.0001*, GA vs GE, P=0.0001* | GB vs GC, P=0.001*, GB vs GD, P=0.005*, GB vs GE, P=0.007*, GB vs GF, P=0.009* | - | - | - | - |

*Significant (P<0.05)

Total WBC significantly increased in subchronic exposed groups with normal or sensitized lungs, also in sensitized acute exposed lungs which suggest that RSBP had the potential to alter host pulmonary immune defense mechanisms. However, gp B, which was sensitized and not exposed to RSBP, also had an increase of WBC, once sensitization is related to inflammation. So, cell recruitment observed in groups E and F could have happened in response to both sensitization and RSBP exposure.

In the present study, non exposed Egg albumin sensitized lungs (gp B), acute normal RSBP exposed (gp C), (gpE) acute RSBP sensitized and subchronic RSBP sensitized (gp F) exposed lungs show significant decrease in macrophages count compared to control non exposed group. This may be explained by another study of Nieuwenhuizen et al. (2012) who demonstrated that macrophages are not necessary for allergic airway disease, and may only be a consequence of the elevated T helper 1-lymphocytes (Th2) response. They studied the contribution of macrophages to acute, chronic and house-dust-mite-induced allergic airway inflammation by using mice with abrogated IL-4Rα signaling on macrophages. It was demonstrated that airway hyperreactivity, Th2 responses, mucus hypersecretion, number of eosinophils, and collagen deposition were not significantly affected by decreased development of macrophages. Also, macrophages seem to be beneficial to the resolution of asthma through production of IL-10 but are not present or not functional in asthma, and therefore allergic inflammation can progress (Fitzpatrick et al., 2010). This of course is in line with the above cited finding by Shaykhiev et al. (2009) that macrophages genes are down regulated in alveolar macrophages of healthy smokers and smoking COPD patients as compared to nonsmokers.

It has been noticed that the increase of Neutro-
Figure 1. Lung section of guinea pig with normal lung (gp A) aspirated normal air showing normal lung architecture except some minimal perivascular and peribronchial mononuclear cellular infiltration (single arrow head) with normal interalveolar septa (double arrow head) and alveolar spaces (in five guinea pigs out of six) (H& E ×125).

Figure 2. Lung section of guinea pig with sensitized lung aspirated air atmosphere (gp B) showing centrilobular emphysematous changes (single arrow head) with thickened bronchial walls (star) (three guinea pigs out of six) (H& E ×125).

phils count occurred not only in RSBP groups (D, E, F), but also in Group B (Sensitized only) compared to control non exposed (gp A). This suggests that the sensitization of the lungs and exposure of RSBP for two or four weeks induce proliferation of neutrophillls and infiltration in the lung. Also, we found a close association of neutrophil infiltration with emphysematous changes, and destruction of alveolar walls (Figures 1- 9). These findings indicate that the protease-antiprotease imbalance had already occurred in exposure model. In
other studies, an increase of neutrophils in the airway lumen has been documented by bronchoalveolar (BAL) fluid and sputum analysis in smokers with mild to moderate chronic airway obstruction (Bohadana et al., 2004), and a good correlation was observed between the number of neutrophils and the annual decline in forced
expiratory volume in the first second (FEV1) (Bohadana et al., 2004; Ravinder et al., 2012).

It has been noticed that eosinophils was not present in the BAL of control (gpA). It has been noticed that the increase of eosinophils count occurred in sensitized groups exposed or not. The significant increase was only present in sub chronic exposure of RSBP (gpF) compared to nonexposed sensitized group (gpB). Airway inflammation with eosinophilic infiltration of the bronchialmucosa is a characteristic feature of atopic asthma (Akuthota et al., 2011). It have been found that stimulation ofmacrophages by ovalbumin uptake induced increased production of IL-10 by these macrophages which could play an important role in the resolution of asthma (Fitzpatrick et al., 2010), and this resulted in lower levels of IL-5 and ovalbumin-specific IgE, and a lower number of eosinophils in a mouse model of asthma (Vissers et al., 2004).

It has been reported that rice straws, and its smoke up regulate the expression of intercellular adhesion molecule-1 (ICAM-1) and human leucocyte antigen (HLA) on Eosinophils which play an important role in Eosinophils function, migration and degranulation (Kayaba et al., 2004). Eosinophils may also contribute to the induction of airway remodelling by synthesizing a variety of profibrotic mediators. Eosinophils are thought to be an important source of the potent pro-fibrotic cytokine TGF-β (Cho et al., 2004). TGF-β is able to induce extracellular matrix (ECM) protein production (Kenyon et al., 2003), and also contributes to the accumulation of fibroblasts below the reticular basement membrane by stimulating fibroblast proliferation. It further contributes to airway remodelling by promoting the differentiation of myofibroblasts from resident fibroblasts, and also from circulatingprecursor cells known as fibrocytes (Mori et al., 2005). The differentiation of myofibroblasts into smooth muscle cells (Wicks et al., 2006), and their proliferation (McMillan et al., 2005) may also be governed by TGF-β. This was confirmed by histopathological examination of lungs sample of subchronic sensitized RSBP exposed group (gpF) showed pericatricial emphysema with interstitial fibrosis in three animals out of six (Figure 12). In the present study, it has been noticed that basophiles wasn't present in the BAL of all groups except in acute and subchronic RSBP exposed sensitized lungs with no significant changes. Histopathological examination of lungs sample of acute RSBP exposed group (gp C) showed mild to moderate thickened interalveolar septa (mild edema and minimal infiltrated) together with mild to moderate peribronchial mononuclear cellular infiltrations normal empty alveolar spaces in four animals (Figure 5), and the two remaining animal showed the same findings associated with intraalveolar cellular exudates (Figure 6). These findings are compatible with previous study (Li et al., 2003).

Histopathological examination of lungs sample of subchronic RSBP showed the result of exposed group (gp D). Another two animals showed diffuse thickening of
Figure 6. Lung section of guinea pig with normal lung aspirated RSBP for two weeks (gp C) showing mild to moderate thickened interalveolar septa (arrow head) (mild edema & minimal infiltrated) together with mild to moderate peribronchial mononuclear cellular infiltrations with normal empty alveolar spaces with intraalveolar cellular exudates (star) (in two guinea pigs out of six) (H&E ×125).

Figure 7. Lung section of guinea pig with normal lung aspirated RSBP for four weeks (gp D) showing moderate mononuclear interstitial & prebronchial mononuclear cellular infiltration associated with interalveolar cellular debris (arrow head) with focal areas of destroyed interalveolar septa (emphysematous spaces) (star) (in two guinea pigs out of six) (H&E ×125).
Figure 8. Lung section of guinea pig with normal lung aspirated RSBP for four weeks (gp D) showing showed diffuse thickening of interalveolar septa with mononuclear cellular infiltration around blood vessels and bronchioles (star) with patchy emphysematous spaces (arrow head) (in two guinea pigs out of six) (H&E ×125).

Figure 9. Lung section of guinea pig with normal lung aspirated RSBP for four weeks (gp D) showing peribronchial focal micronodular mononuclear cellular accumulates (arrow head) (in two guinea pigs out of six) (H&E ×125).

interalveolar septa with mononuclear cellular infiltrations around blood vessels and bronchioles with patchy emphysematous spaces (Figure 8), and the last two animals showed peribronchial focal micronodular mononuclear cellular accumulates (Figure 9). These findings are compatible with previous studies (Li et al.,
Figure 10. Lung section of guinea pig with sensitized lung aspirated RSBP for two weeks (gp E) showing interstitial mononuclear cellular infiltration (star) with numerous cellular nodules with large wild emphysematous spaces (arrow head) (in three guinea pigs out of six) (H&E ×125).

Figure 11. Lung section of guinea pig with sensitized lung aspirated RSBP for two weeks (gp E) showing the typical picture of interstitials pneumonia (star) (interstitial mononuclear cellular infiltration with the presence of some hyalinized bodies), dilated arterioles and perivascular edema (arrow head) (in three guinea pigs out of six) (H&E ×125).

2003; Sezer et al., 2007). Histopathological examination of lungs sample of sensitized acute RSBP showed the result of exposed group (gp E). The remaining two animals showed the typical picture of interstitials pneumonia (interstitial mononuclear cellular infiltration with the presence of some hyalinized bodies), dilated arterioles and perivascular edema (Figure 11). Histopathological examination of...
Figure 12. Lung section of guinea pig with sensitized lung aspirated RSBP for four weeks (gp F) showing interstitial and peribronchial fibrosis (arrow head) with emphysema (star) (in three guinea pigs out of six) (H&E ×125).

Figure 13. Lung section of guinea pig with sensitized lung aspirated RSBP for four weeks (gp F) showing dense interalveolar inflammatory infiltration hyperplastic (arrow head) bronchial epithelium with peribronchial inflammatory infiltration with peribronchial emphysematous bolea (star) formation (in three guinea pigs out of six) (H&E ×125).

lungs sample of sensitized subchronic RSBP exposed group (gp F) showed pericatricial emphysema with interstitial fibrosis in three animals (Figure 12). Histopathological examination using H&E stain of lungs sample of sensitized subchronic RSBP exposed group (gp F). The remaining three animals showed dense interalveolar inflammatory infiltration hyperplastic bronchial epithelium with peribronchial inflammatory infiltration with peribronchial emphysematous bolea formation (Figure 13). These findings are compatible
Figure 14. Mean values of malondialdehyde (MDA) and nitric oxide content (NO) of the study groups of guinea pigs (exposed and not exposed to rice straw burning products (RSBPs) (n=36). Group A: control group, group B: Egg albumin sensitization group, group C: Acute RSBP exposed group, group D: Subchronic RSBP exposed group, group E: Sensitized acute RSBP Exposed group, group F: Sensitized Subchronic RSBP exposed group. Results expressed as The mean of MDA content (µM/g tissue) ± SD; n = 6/group. The mean value was significantly different between GA and GD (normal lungs) at p < 0.05. The mean value was significantly different between GB and GE, GF (sensitized lungs) at p < 0.05.

Figure 15. Mean values of total white blood cells (TWBCs) of the study groups of guinea pigs (exposed and not exposed to rice straw burning products (RSBPs) (n=36). Group A: control group, group B: Egg albumin sensitization group, group C: Acute RSBP exposed group, group D: Subchronic RSBP exposed group, group E: Sensitized acute RSBP Exposed group, group F: Sensitized Subchronic RSBP exposed group. Results expressed as the mean of total leukocytic count (cell/μm³) ± SD; n = 6/group. The mean value was significantly different between GA and GD (normal lungs) at p < 0.05. The mean value was significantly different between GB and GE, GF (sensitized lungs) at p < 0.05.
Figure 16. Mean values of macrophages of the study groups of guinea pigs (exposed and not exposed to rice straw burning products (RSBPs) (n=36). Group A: control group, group B: Egg albumin sensitization group, group C: Acute RSBP exposed group, group D: Subchronic RSBP exposed group, group E: Sensitized acute RSBP Exposed group, group F: Sensitized Subchronic RSBP exposed group. Results expressed as the mean of macrophages count (cell/um$^2$) ± SD; n = 6/group. The mean value was significantly different between GA and GC (normal lungs) at p < 0.05.

Figure 17. Mean values of neutrophils of the study groups of guinea pigs (exposed and not exposed to rice straw burning products (RSBPs) (n=36). Group A: control group, group B: Egg albumin sensitization group, group C: Acute RSBP exposed group, group D: Subchronic RSBP exposed group, group E: Sensitized acute RSBP Exposed group, group F: Sensitized Subchronic RSBP exposed group. Results expressed as the mean of neutrophils count (cell/um$^2$) ± SD; n = 6/group. The mean value was significantly different between GA and GD (normal lungs) at p < 0.05. The mean value was significantly different between GB and GE, GF (sensitized lungs) at p < 0.05.

Malondialdehyde (MDA) is the end product of lipid peroxidation, and an indicator of oxidative stress (Kamal et al., 1989). Antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are essential for preservation of cellular balance and...
elimination of free radicals (McLaughlin et al., 1990). The present results have shown that there was significant increase in the level of MDA in the lung tissues in (D, E, F) groups that had been exposed to the smoke of RSBP in normal or sensitized lungs. These results suggest that the RSBP exposure has a potent potential capacity for being a source of reactive oxygen species and possibly oxidizing species. Confirmatory observation by Ho and Kou (2002) who reported the increase hydroxyl radical (OH) burdens following smoke exposure which was actively involved in evoking the acute irritant effects of wood smoke. Oxidative stress has been shown to be an important contributor to the pathogenesis of COPD (Ito et al., 2009).

It has been demonstrated in the present study that the concentration of nitric oxide (NO) contents showed a significant increase in the normal group than in other RSBP exposed groups. It have been reported that NO, was significantly decreased in cigarette smoke-exposed animals compared with healthy controls (Pekmez et al., 2010). It is quite possible that RSBP exposure was interfered with the function of endothelial nitric oxide synthase (eNOS). This was supported by the observation of MacNee (2000) who reported that smokers have serum and tissue evidence of oxidative damage to many proteins like tetrahydrobiopterin (BH4), a required co-factor for eNOS activity, which can be altered to its inactive BH2 form by oxidants, and in this situation switches production from NO to superoxide (O⁻) (Heitzer et al., 2000; Wagnner et al., 2007). Peroxynitrite, a highly reactive molecule, is produced in a near diffusion-limited rate as a reaction between O₂⁻ and NO. Peroxynitrite can oxidatively inactivate eNOS, thus decreasing NO synthesis, and in addition peroxynitrite catalytically disrupts eNOS, resulting in increased O₂⁻ production by the eNOS dimers (Zou et al., 2004).

Furthermore, cigarette smoke has been shown to inhibit production of tetrahydrobiopterin (Heitzer et al., 2000), disrupt the active eNOS dimers, and abnormally phosphorylate eNOS, producing an inhibitory state, all of which are alterations that would reduce NO bioavailability (Wagnner et al., 2007). Smoke also exerts a number of other effects on eNOS production. TNFα which is increased in the sputum of human smokers with chronic obstructive pulmonary disease (COPD) and in the plasma of animal models of COPD (Churg et al., 2002), destabilizes eNOS mRNA (Neumann et al., 2004; Searles, 2006). Smoke also contains large concentrations of O₂⁻ which can reduce functional levels of NO by converting it to peroxynitrite (Wright and Churg, 2008).

**CONCLUSION**

This study has demonstrated that RSBP induced pulmonary emphysematous lesions are progressive with subsequent smoke exposures together with the sensiti-

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**Figure 18.** Mean values of eosinophils and basophils of the study groups of guinea pigs (exposed and not exposed to rice straw burning products (RSBPs) (n=36). Group A: control group, group B: Egg albumin sensitization group, group C: Acute RSBP exposed group, group D: Subchronic RSBP exposed group, group E: Sensitized acute RSBP Exposed group, group F: Sensitized Subchronic RSBP exposed group. Results expressed as the mean of Eosinophils count (cell/μm²) ± SD; n = 6/group. The mean value was significantly different between GB and GF (sensitized lungs) at p < 0.05. Results expressed as the mean of Basophils count (cell/μm²) ± SD; n = 6/group.
ization of the lung in the present model. RSBP have the ability to change the counting of macrophages, neutrophils, eosinophils in the BAL of guinea pigs lungs compared to normal lavages. RSBP exposure has a potent potential capacity for being a source of reactive oxygen species and possibly oxidizing species which can lead to decrease nitric oxide content in lung tissue. It was found out that Rice straws burning products can easily penetrate the pulmonary alveoli and damage the health of humans. It is recommended that the rice burning activities should be stopped as it affects human health, and we should find alternative ways for disposal of the rice after harvesting.

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Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES


method for simultaneous detection of nitrate and nitrite. Nitric oxide
air quality during wheat and rice crop stubble burning episodes in
Patiala. Atmos. Environ. 43:239-244.
contribute to the myofibroblast population in wounded skin and
Pekmez H, Ogeturk M, Ozyurt H, Sonmez MF, Colakoglu N, Kus I
(2012). Smoking-dependent reprogramming of alveolar macrophage polarization: implication for
protein kinase C. Endothelium 14:245-255.
Enhanced upregulation of smooth muscle related transcripts by TGF
beta2 in asthmatic (myo) fibroblasts. Thorax 61:313-319.
Wright JL, Churg A (2008). Short-term exposure to cigarette smoke
induces endothelial dysfunction in small intrapulmonary arteries: analysis using guinea pig precision cut lung slices. J. Appli.
104:1462-1469.
Xia T, Korge P, Weiss JN, Li N, Venkatesen MI, Sioutas C, Nel A
(2004). Quinones and aromatic chemical compounds in particulate
matter induce mitochondrial dysfunction: implications for ultrafine
identification and size distribution of atmospheric polycyclic aromatic
hydrocarbons during rice straw burning period. Atmos. Environ. 40:
1266-1274.
residue burning in the field and its influence on ambient air quality in
Yoshioka T, Kawada K, Shimada T, Mori M (1979). Lipid peroxidation in
360:376-386.
Effects of exposure to rice-crop residue burning smoke on pulmonary
functions and Oxygen Saturation level of human beings in Patiala
Regalado J, Pérez-Padilla R, Sansores R, Páramo Ramirez JI, Brauer
respiratory symptoms and lung function in rural Mexican women. Am.
Searles CD (2006). Transcriptional and posttranscriptional regulation of
Physiol. 291:803-816.
Caffeic Acid Phenethyl Ester on the Histopathological Changes in the
Shaykhiev R, Krause A, Salit J, Strulovici-Barel Y, Harvey BG,
183(4):2867-2883.
Tippayawong D, Kim Oanh NT (2007). Effects from open rice straw
burning emission on air quality in the Bangkok Metropolitan Region.
factor: documentation for AP-42, open burning. In: Office of Air
Quality Planning and Standards and Office of Air and Radiation (Ed.),
AP-42 Database. US Environmental Protection Agency, North
Carolina p 34.
Viana M, López JM, Querol X, Alastuey A, García-Gacio D, Blanco-
Heras G, López-Mahía P, Piñeiro-Iglesias M, Sanz MJ, Sanz F,
Chi X, Maenhaut W (2008). Tracers and impact of open burning of
rice straw residues on PM in Eastern Spain. Atmos. Environ.