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Full Length Research Paper

HRCT findings in rats with long-term exposure to smokes of cigarette and biomass fuels

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The harmful effects of tobacco's smoke have been well-known for years and several studies revealed that exposure to biomass smoke can markedly increase the prevalence of respiratory disorders. In this study, separate and combined effects of tobacco smoke and biomass exposure on pulmonary radiologic findings were examined in rats. The subjects were divided into four groups; control group, exposure group (tobacco smoke alone), exposure group (biomass smoke alone) and exposure group (both tobacco and biomass smoke). High-resolution computed tomography examinations were performed. Presence or absence of radiological abnormalities such as the ground-glass appearance, fibrotic band, peribronchovascular thickening, air-cyst bullae, pleural thickening, bronchiectatic change and curvilinear densities were recorded. Many radiological findings were more prominent in groups exposed to smoke. This study demonstrates harmful effects of tobacco and biomass smoke on lungs in a rat model.

Key words: Animal study, biomass smoke, thoracic computed tomography, tobacco smoke.

INTRODUCTION

The harmful effects of tobacco smoke have been well-known for years. Moreover, these effects have been demonstrated by experimental trials (Desai et al., 2003). Organic materials such as charcoal, dried dung and firewood, which are derived from plant residues and used as fuel, are collectively named as biomass. In developing countries, biomass fuel is burned to obtain energy for daily necessities like heating and cooking. Biomass fuel smoke contains a number of noxious materials such as carbon monoxide (CO), nitric oxide (NO), sulphur oxides (SOx), formaldehyde, polycyclic organic matter (POM), and benzopyrene. It was reported that more than 500 million people were exposed to smoke and noxious molecules emitted from burning these fuels (WHO, 1999). Several studies revealed that exposure to biomass smoke can markedly increase the prevalence of respiratory disorders (Behera and Jindal, 1991; Perez-Padilla et al., 1996; Albalak et al., 1999; Bruce et al., 1998).

Our clinic was the first center to draw attention to relatively high frequency of COPD in women who never smoked, but were exposed to biomass fuels in Turkey (Demirtas et al., 1999). The potential mechanism of impact and radiological and functional effects of biomass exposure were also investigated (Gani et al., 2000; Sungu et al., 2001; Kara et al., 2003; Arslan et al., 2004). Also, recently, a case-control study showed the importance of biomass and tobacco smoke exposures in the development of COPD (Sezer et al., 2006). It is suspected that the accompaniment of passive smoking and biomass exposure might have implications other than COPD. Although a few experimental studies are published, it is hard to claim that information on this issue is clear enough (Fidan et al., 2006; Ozbay et al., 2009). Furthermore, current literature does not provide sufficient data illuminating the possible chronic effects of tobacco smoke and biomass exposures on respiratory apparatus. This inspired us to conduct a study in order examine the separate and combined effects of tobacco smoke and biomass exposure on pulmonary radiologic finding in rats.

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Figure 1. Biomass exposure of rats.

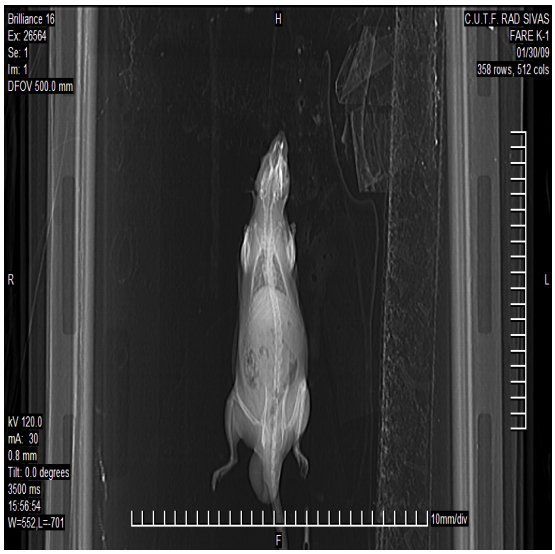


Figure 2. Full body CT scan of a rat.

MATERIALS AND METHODS

Experimental animals

Twenty-eight adult male Wistar-strain albino rats, each weighing about 300 g, were obtained from the laboratories of Experimental Animals Research and Practice Center, Cumhuriyet University, Sivas. The animals were housed in 920 cm² floor area plastic cages (Type III, Allentown Inc., USA) under standard laboratory conditions (illuminated between 7:00 a.m. and 8:00 p.m., temperature of 21 ± 2°C, and relative humidity of 55%) and maintained according to the recommendations in the 'Guide for Care and Use of Laboratory Animals' of the National Institutes of Health (NIH, 1986). The animals, after being exposed to tobacco or biomass smoke, they were taken to another continuously ventilated room.

The study was initiated with four groups consisting of eight rats each. Two rats from Group IV, one rat from Group III and another one from Group II died of unknown causes. At the end of the study, one rat from the control group was excluded randomly and thereby the groups were equalized for number of subjects.

Four final groups were established as:

1. Group I: control group (no exposure to tobacco or biomass smoke),
2. Group II: exposed to tobacco smoke,
3. Group III: exposed to biomass smoke,
4. Group IV: combined exposure to tobacco and biomass smoke.

Group I was kept under standard laboratory conditions with ventilation and was not exposed to any kind of smoke.

Exposure to tobacco smoke

The rats in Group II were taken to another room every morning between 9:00 and 10:00 for 6 months. Smoke from 5 burning cigarettes was pumped into this ventilated room for 1 h a day. Every cigarette was kept burning for about 15 - 20 min. Meanwhile, ambient CO concentration was measured to reach 116 ± 23 ppm and oxygen saturation was measured as 20% with the use of GT series gas detector (Gas Measurement Instruments, UK) (Wright et al., 1992; GMI, 2007).

Exposure to biomass smoke

The rats in Group III were taken to another room every morning between 9:00 and 10:00 for 6 months. In this ventilated room, the smoke produced by burning about 500 g of dried animal dung was dispersed into the environment with a manual bellows modified from that used in previous studies (Figure 1). In the meantime, detected values of ambient CO concentration and oxygen saturation were 228 ± 30 ppm and 20%, respectively (Wright et al., 1992; GMI, 2007).

In the same manners described above, the rats in Group IV were exposed to tobacco smoke between 11:00 a.m. and 12:00 a.m. and to biomass smoke between 14:00 p.m. and 15:00 p.m. every day for 6 months. Meanwhile, ambient CO concentration was detected as 116 ± 23 ppm and oxygen saturation was measured as 20% (Wright et al., 1992; GMI, 2007).

According to United States Environmental Protection Agency, standard level for 8-h average of carbon monoxide concentrations is 9 ppm; on the other hand, it was reported that this level could reach 10 - 500 ppm during cooking (USEPA, 1997). In our study, the carbon monoxide levels in the exposure setting were appropriate to these parameters.

At the end of sixth week, the rats underwent thoracic computerized tomography scan under general anesthesia (Figure 2).

Computed tomography studies

High-resolution computed tomography examinations were performed with a 16-slice Brilliance CT scanner (Philips, Cleveland, Ohio). The CT images were obtained with 1-mm slice thickness. The other scan parameters were 120 kV, 30 mA. The images were examined with -300 to -700 and 1600 - 2000 Hounsfield Unit (HU) window width and levels. All of the CT scans were performed in supine positions and no contrast medium was used. Two observers evaluated the CT examinations. Presence or absence of radiological abnormalities such as the ground-glass appearance, fibrotic band, peribronchovascular thickening, air-cyst bullae, pleural thickening, bronchiectatic change, curvilinear densities were recorded.

Statistical analysis

CT findings were recorded as present (+) or absent (-). Statistical analysis of the data was done by chi-square test using SPSS software program. Statistically, p value of less than 0.05 was accepted as significant.

RESULTS

Ground-glass appearance

The presence of ground-glass appearance was 100% in group IV, 28.6% in group II, 57.1% in group III, and 0% in group I. The differences between groups IV and II, IV and I, and III and I were statistically significant ($p < 0.05$), but no difference was found between groups IV and III, II and III, and II and I ($p > 0.05$).

Fibrotic bands

The fibrotic band presence was 83.3% in group IV, 85.7% in group II, 71.4% in group III, and 14.3% in group I. The differences between groups IV and I, II and I, and III and I were statistically significant ($p<0.05$), but no difference was found between groups IV and II, IV and III, and II and III ($p>0.05$).

Peribronchovascular thickening

The presence of peribronchovascular thickening was 83.3% in group IV, 71.4% in group II, 57.1% in group III, and 0% in group I. The differences between groups IV and I, II and I, and III and I were statistically significant ($p < 0.05$), but no significant difference between groups IV and II, IV and III, and II and III ($p > 0.05$) was observed.

Air cyst-bullae

The occurrences of air cyst-bulla were 66.7% in group IV, 42.9% in group II, 28.6% in group III, and 14.3% in group I. There was no significant difference between any of the groups ($p > 0.05$).

Pleural thickening

The presence of pleural thickening was 33.3% in group IV, 0% in group II, 42.9% in group III and 0% in group I.

There was no significant difference between any of the groups ($p > 0.05$).

Bronchiectatic changes

The presence of bronchiectasis was 33.3% in group IV, 14.3% in group II, 28.6% in group III and 0% in group I. There was no significant difference between any of the groups ($p > 0.05$).

Curvilinear densities

Curvilinear densities were observed in 66.7% of group IV, 57.1% in group II, 100% in group III, and 14.3% in group I. The difference between groups III and I was significant ($p < 0.05$), but no significant difference was observed between other groups ($p > 0.05$).

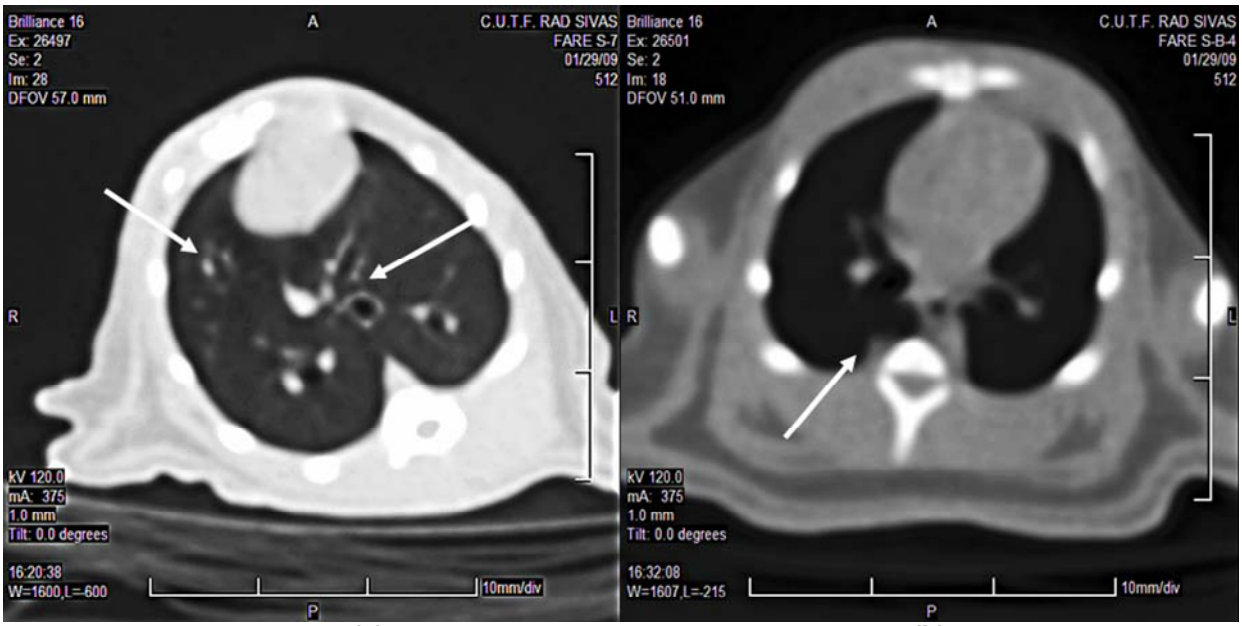
DISCUSSION

This study's data have shown that the presence of ground-glass appearance was 100% in group IV (Figure 3(a)), 28.6% in group II, and 57.1% in group III. The fibrotic band presence was 83.3% in group IV (Figure 3(b)), 85.7% in group II, 71.4% in group III, and 14.3% in group I and the presence of peribronchovascular thickening was 83.3% in group IV (Figure 4(a)), 71.4% in group II, 57.1% in group III. The occurrences of air cyst-bulla were 66.7% in group IV, 42.9% in group II, 28.6% in group III and the presence of pleural thickening was 33.3% in group IV, 0% in group II, 42.9% in group III (Figure 4(b)). The presence of bronchiectasis was 33.3% in group IV, 14.3% in group II, 28.6% in group III and curvilinear densities were observed in 66.7% of group IV, 57.1% in group II, 100% in group III (Table 1).

This research evaluated laboratory animal models computed for tomography changes caused by cigarette and biomass inhalation, and was inspired by the lack of animal models based on both cigarette smoke and biomass fuels.

Biomass fuel is any material derived from plants or animals which is burnt by humans. In the central Anatolia, the use of animal dung and crop residues is widespread. In central Anatolia, most of the people live in the rural areas, where about 80% of households rely on biomass fuels as their major or only source of domestic energy for cooking and sometimes domestic heating. The wide-spread use of biomass fuels indoors leads to several levels of pollution that are among the highest, ever measured. Since people in rural areas of Turkey spend so much time in cooking daily, exposure to these high levels is considerable, especially among women and children. The biofuels are usually burnt in open fires or simple stoves (Kara et al., 2003; Arslan et al., 2004; Ekici et al., 2005).

Kara et al. (2003) evaluated pulmonary changes with HRCT in a symptomatic and asymptomatic female population exposed to biomass fuels for a long time, and their findings were compared with an asymptomatic control female population. In that study, 92 non-smoking women were divided into three groups. Group 1 consisted of 32 women and their ages which ranged from



(a) **(b)**

Figure 3. Peribronchial thickening and nodular pleural thickening are shown with arrows.

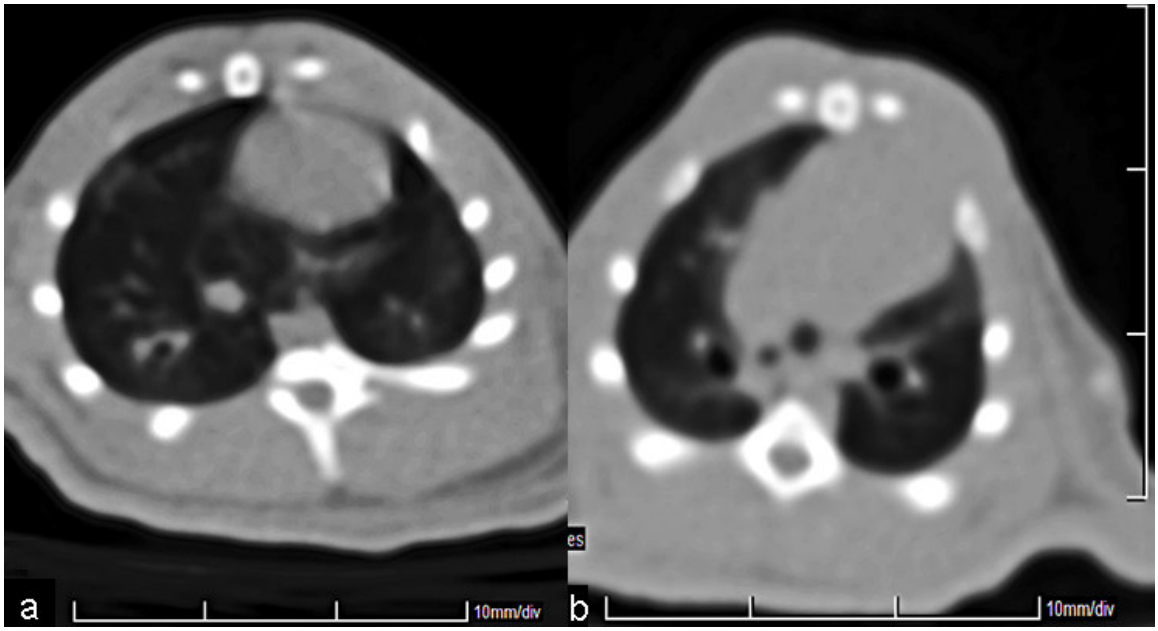


Figure 4. Bilateral ground-glass appearance and fibrotic bands are shown in HRCT images.

24 - 65 years. They concluded that exposure to biomass fuels is likely to cause many lung diseases from interstitial lung diseases to COPD. Their results suggested that in all stages of the disease, pulmonary parenchymal changes due to biomass exposure can be properly evaluated, followed by the HRCT examination. Arslan et al. (2004) conducted an investigation to assess the effects of the biomass fuel exposure on the respiratory system by the radiological findings of the high resolution computed tomographic and spirometric parameters including the diffusion capacity (at rest for carbon monoxide) and single breath diffusion lung capacity (for carbon monoxide). Their series included 21 consecutive non-smoker women, using biomass for cooking and heating

Table 1. Comparisons of the high-resolution computed tomography (HRCT) findings of the groups.

	GGA		FB		PBVT		ACB		PT		BC		CD	
	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Group IV	0	6	1	5	1	5	2	4	4	2	4	2	2	4
n (%)	(0)	(100)	(16.7)	(83.3)	(16.7)	(83.3)	(33.3)	(66.7)	(66.7)	(33.3)	(66.7)	(33.3)	(33.3)	(66.7)
Group III	3	4	2	5	3	4	5	2	4	3	5	2	0	7
n (%)	(42.9)	(57.1)	(28.6)	(71.4)	(42.9)	(57.1)	(71.4)	(28.6)	(57.1)	(42.9)	(71.4)	(28.6)	(0)	(100)
Group II	5	2	1	6	2	5	4	3	7	0	6	1	3	4
n (%)	(71.4)	(28.6)	(14.3)	(85.7)	(28.6)	(71.4)	(57.1)	(42.9)	(100)	(0)	(85.7)	(14.3)	(42.9)	(57.1)
Group I	7	0	6	1	7	0	6	1	7	0	7	0	6	1
n (%)	(100)	(0)	(85.7)	(14.3)	(100)	(0)	(85.7)	(14.3)	(100)	(0)	(100)	(0)	(85.7)	(14.3)
CWG	$p < 0.05$*		$p < 0.05$*		$p < 0.05$*		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p < 0.05$*	
Group IV - II	$p < 0.05$*		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
Group IV - III	$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
Group IV - I	$p < 0.05$*		$p < 0.05$*		$p < 0.05$*		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
Group II - III	$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
Group II - I	$p > 0.05$		$p < 0.05$*		$p < 0.05$*		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
Group III - I	$p < 0.05$*		$p < 0.05$*		$p < 0.05$*		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p < 0.05$*	

GGA: ground-glass appearance, FB: fibrotic band, PBVT: peribronchovascular thickening, ACB: air-cyst bullae, PT: pleural thickening, BC: bronchiectatic change, CD: curvilinear density. CWG: comparison within the groups, * statistically significant.

and heating at least for 10 years. They found that biomass fuel exposure caused obstructive and restrictive spirometric impairments. They concluded that the high resolution computed tomography and spirometric findings were due to biomass fuels exposure, they presented a particular situation and these findings could be the signs of the “biomass lung”. In this experimental study, especially the HRCT findings were in line with these two last studies.

A large number of studies have reported the associations between indoor biofuel air pollution in developing countries and chronic obstructive lung disease in adults, and acute lower respiratory infection in children. It is estimated that around 50% of the world's population and 75% of those living in developing countries, rely on biomass fuels (wood, dung and crop residues) for cooking and heating (Smith, 1996; Norboo et al., 1991; Pandey, 1984; Pandey et al., 1989).

The fuels used for cooking contain main respiratory irritant smoke particles, including nitro-oxides, sulfur di-oxide, and non-inflammable hydrocarbons. These smoke particles cause chronic bronchitis and air obstructions (Behera and Jindal, 1991).

Among various risk factors, the most important is cigarette smoking for chronic bronchitis and emphysema in males and females in industrialized countries (Masironi, 1984; Masironi and Rothwell, 1988). However, for women living in many developing countries, exposure to indoor pollutants produced by inefficient biomass stoves may be a major contributor to the global burden of disease. Several recent papers have dealt with the subject, including a study conducted in Central Anatolia, Turkey, and many models have been proposed to characterize exposure to respirable particulate matter and carbon monoxide from biomass combustion for the purposes of health effect assessment (Cetinkaya et al., 2000; Smith and Sundell, 2002; Ezzati and Kammen, 2001; Balakrishnan et al., 2002; Naeher et al., 2001; Bruce et al., 2004; Perez-Padilla et al., 1999).

Smoking cigarette is the major cause of chronic obstructive pulmonary disease. In these patients, two respiratory conditions are most closely associated with cigarette smoking: chronic bronchitis and emphysema. Li et al. (2003) evaluated the histological appearance of the lung and measured the levels of vitamin A (retinol) in the lung and serum after smoke exposure in comparison with air-exposed controls. Their experimental group was exposed to cigarette smoke from 20 unfiltered commercial cigarettes per day for 5 days a week, whereas the control group was exposed to air. They concluded that cigarette smoke induces vitamin A depletion in the serum, lung and liver, and that the compromised status of vitamin A is associated with the development of emphysema, as shown by an inverse correlation between lung vitamin A and the development of emphysema.

Fidan et al. (2006) conducted an investigation to compare the effects of cigarette smoke and dried dung smoke exposure on lung histopathology. In that study, three groups each of which included five rabbits were formed. The cigarette smoke group was exposed to cigarette smoke, the biomass group was exposed to dried dung smoke and the control group was exposed to dry air during 1 h daily for 1 month. At the end of 1 month,

animals were sacrificed and lung tissues were examined histopathologically. They concluded that dried dung smoke had severe histopathological effects on the lungs of rabbits.

In conclusion, the problem of the adverse health effects due to biomass exposure and smoking cigarette are complex and are gaining widespread. This is the first study in literature that shows toxic gas inhalation leading to pulmonary parenchymal changes in rats, which may be irreversible and progressive to fibrosis. Therefore, we believe that active and passive smoking must be prohibited in the world, and non-polluting fuels must be used in indoor areas.

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