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Effects of Inhalation of Iron Emission Particles on Some Lung Cellular Parameters in Mice

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Abstract.- Animal toxicological and occupational studies are imperative to assess the health effects of particulate matter (PM) inhalation. The present study was conducted in Wazirabad, a city about 100 Km away toward north west of Lahore. The study area was located within the vicinity of a number of units of small industries involved in manufacturing of scissors and knives etc. During the processing, iron particulate matter (IPM) of varying sizes is emitted profusely when the items are whetted on electric motor driven grindstones. The workers continuously inhale the IPM and further they do not use any type of respiratory mask. Twelve adult mice were kept in an open area surrounded by such whetting units for 10 weeks. During the experimental period the mice were provided with feed and water *ad libitum*. At night when the whetting units stopped working the mice were shifted to a room. A group of mice kept in animal house of the Department of Zoology. University of the Punjab, Lahore served as control. At the end of experiments the mice were anaesthetized and dissected out for obtaining different organs. Lungs were fixed in Bouin's fixative and processed for routine histological analyses. It was revealed that following inhalation of IPM for 10 weeks, nuclei as well as cell's sizes of lungs of the experimental animals decreased as compared to the controls. More over, number of bi/ multinucleate cells in the exposed animals increased over 100% than the control values. Similarly when the tissue sections were stained for IPM over 80 folds more iron particles deposits were observed in the experimental group vs. control animals. These observations suffice to alarm speculating the lung health status of the workers. Occupational studies are urgently required and waiting the attention of public health authorities in the study area.

Key Words: Air pollution, Atmospheric suspended particles, lungs damage.

INTRODUCTION

Particulate matter (PM) in ambient air represents a complex mixture containing particles of different sizes and chemical compositions. However, in urban environments especially those associated with industrial activities, prevailing aerial PM are indicative of their local origins. Assessing the effects of airborne PM in appropriate animal models is critical in understanding the ways through which they may exert adverse health effects in humans (Kato *et al*., 2000; Miller, 2000; Vincet, *et al*., 2001; Kato *et al*., 2003; Katterman *et al*., 2007). Scientific and regulatory communities are concerned about potential neoplastic and non neoplastic health effects of inhaled particles in humans (Mauderly *et al*., 1994; Nikula, *et al*., 2001; Sydbom *et al*., 2001).

Warheit *et al*. (1997) studied the effects of inhalation of high concentration of low toxicity dusts in rats at several post exposure time periods and demonstrated that exposure to high dust concentrations of two different innocuous particles titanium dioxide and carbonyl iron produced sustained pulmonary inflammation and enhanced proliferation of pulmonary cells. These workers also noticed impairment of particle clearance, deficits in macrophage function, and the appearance of macrophage aggregates at sites of particle deposition.

Nikula *et al*. (2001) have described that much of the experimental data on chronic health effects of inhaled particles come from rats. Considerable efforts have been expended in measuring and modeling pulmonary deposition of inhaled particles in rodents and other species (Brody *et al*., 1983; Adamson *et al*., 1999; Miller, 2000; Fernandez *et al*., 2002; Kodavanti *et al*., 2002; Kodavanti *et al*., 2003; Mossman *et al*., 2007). Being critical constituents of ambient particulate matter, transition metals such as iron may play an important role in health outcomes associated with air pollution.

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Inhalation of iron particles leads to oxidative stress associated with a pro inflammatory response in a dose-dependent manner (Warheit *et al*., 1997; Zhou *et al*., 2003a,b). Recently Antonini *et al*. (2006) have reported that the respiratory effects in welders appear in terms of lung function changes, metal fume fever, bronchitis and a possible increase in the incidence of lung cancer.

The present study was conducted in city Wazirabad of Pakistan that is famous for manufacturing of scissors, knives and various other associated items. Twelve adult mice were kept within the vicinity of a number of the whetting units. The mice were kept in a standard animal cage and provided with food and water supplies *ad libitum*. After an exposure of ten weeks to the air borne metal particulate matters, which had been inhaled also by the whetting workers, the animals were studied for histological changes in their lungs. A comparable group of mice kept in Lahore about 100 Km away from the study area served as control. Microscopic analysis of the exposed animals indicated significant amounts of deposition of iron particulate matter within the lung cells accompanied by a number of histological changes. These results are sufficient to speculate what might had been happening with the human lungs of the resident of the study area, especially the whetting workers.

MATERIALS AND METHODS

Twelve adult male mice were housed in a standard animal cage facility provided with food and drinking water *ad libitum*. The cage was kept daily in the morning in an open area surrounded by several whetting units involved in manufacturing of scissors and knives etc. At the evening when the whetting units stopped working, the cage was shifted to a room till the next morning .The animals were exposed to the experimental area for 10 weeks. Iron particulate matter of varying sizes is emitted profusely when the items are whetted on electric motor driven grindstones. A group of mice kept in animal house of the Department of Zoology University of the Punjab, Lahore served as control. At the end of experiments the mice were anaesthetized and dissected out for obtaining different organs. Lungs were fixed in Bouin's fixative and processed for histological analysis, routinely.

The tissue sections 8 μm thick were stained with Harris's Hematoxylin for 5 minutes and then washed in tap water. Excess stain was removed with 1% acid alcohol. Slides were again rinsed in tap water and counter stained with 1% eosin Y for three minutes. They were again washed with water and dehydrated through graded alcohol. The slides were then cleared in xylene followed by the application of Canada balsam and cover slips (Loughlin, 1993). Slides were then observed under light microscope at 40X and 100X. Length and width of alveolar cells, and their nuclei were measured with the help of an ocular micrometer calibrated to μm. While observing sections of control and experimental lung tissues at 100X numbers of binucleate and multinucleate cells were recorded per unit area.

The tissue sections were also processed for the iron staining (Perl's method). Sections were deparafinized and treated with ferrocyanide solution for 30 to 60 minutes and then washed in distilled water. Sections were then counter stained with 1% aqueous neutral red for 3 minutes followed by washing in tap water. They were dehydrated through graded alcohol, cleared in xylene and mounted as described above (Budavari, 1996). Iron particles were counted at 40X both in the experimental and control tissues.

RESULTS AND DISCUSSION

Haematoxylin and eosin stained sections revealed that following inhalation of iron particulate matter (IPM) for 10 weeks, nuclei as well as cells' sizes from lungs of the experimental animals decreased as compared to the controls. Moreover, number of bi/multinucleate cells in the exposed animals increased over 100% than the control values. (Fig. 1, Table I) Beno *et al*. (2004, 2005) have described that in the experimental assessment of effects of particulate matter, numbers of binucleate cells (BNC) and of multinucleate cells (MNC) in lung cell suspensions may serve a useful biomarker of the inflammation. The present results in the terms of higher numbers of BNC/MNC in the exposed tissues thus clearly indicate the inflamed status of the tissue. It is recommended that clinical

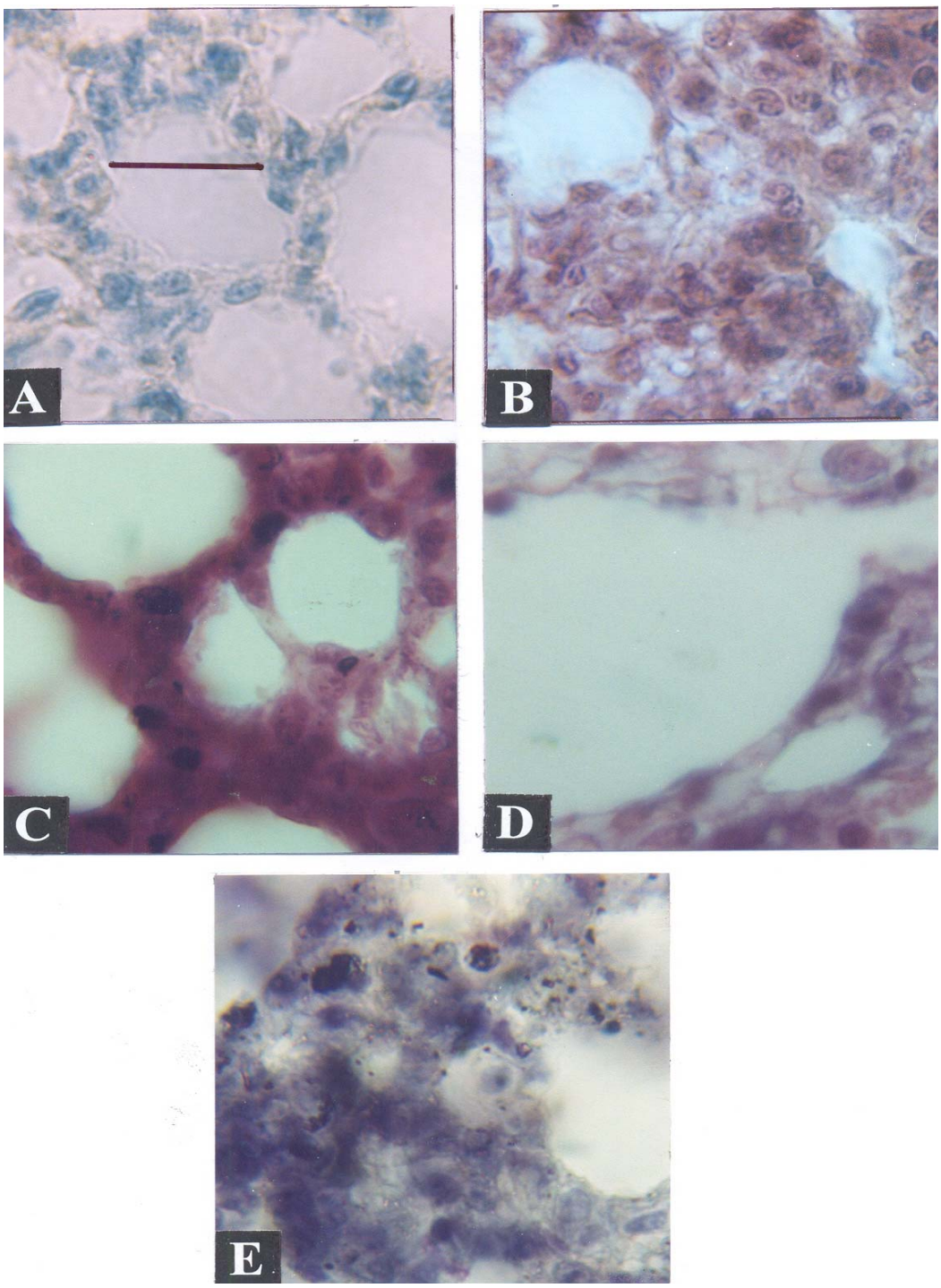

Fig. 1. Histological section of lung of control mouse (A), showing intact nature of epithelial cells and no macrophage infiltration; B, lung of an exposed mice showing abundant infiltration of macrophages and distorted epithelium; C, lung of an exposed mice showing distorted nature of epithelium; D, lung of exposed mice showing distorted epithelium and degenerated cells; and E, lung of exposed mice showing infiltration of macrophages and deposition of particulate matter. A, B, H & E staining; C-E, Perl's staining. All photomicrographs have the same magnification. Bar = 20μm.

Table I.- Sizes (µm) of cells and nuclei, number of multinucleated cells and iron particles deposition/mm^2 of sections of the lungs tissues of control and experimental mice.

Experiment	Cells		Nuclei		Multinucleate cells	Particle counting
	Length	Width	Length	Width		
Control	13.077±0.582	10.485±0.420	9.300±0.440	8.368±0.200	596.038±23.653	39.691±5.863
Exposed	11.545±0.263*	4.955±0.177**	4.309±0.127**	2.840±0.132**	1563.942±197.423**	1086.361315.753**

Values represent means of four replicates ± S.E.M. For each animal, 10 cells were studied randomly from a representative section. For the multinucleate cells and iron particles three representative sections of each animal were studied. Values with asterisk(s) are significantly different from the corresponding controls.* $p<0.05$; ** $p<0.01$ (Student's t-test)

studies be conducted for the whetting workers. These experimental data are suggestive to consider that the workers exposed for longer period of time in the study area might have developed lungs inflammation.

Tissue sections stained for IPM appear to have 27 folds more iron particle deposits in the experimental group vs control animals (Table I). Ogami *et al*. (2004) have used morphometric point counting method (PCM) for evaluating pulmonary inflammation in the assessment of the biological hazards of the inhaled respirable particles. Morphometric PCM method was used for qualitative analysis of pulmonary inflammation. These workers have considered PCM scoring system, a useful and sensitive tool for qualitatively evaluating the biological hazards of new particle types for which no toxicological information exists for low dose exposure.

Nikula *et al*. (2001) used point-counting method of planimetry to estimate the volume density of particulate material in the lung sections and the volume percentage of the total particulate material in defined anatomic compartments of the lungs. They have reported that 80% of the particulate material retained in alveolar macrophages or extracellularly in parenchymal lumens. These workers have suggested that the retained particulate material may be key to pathologic sequelae, which include acute and chronic inflammation, epithelial hyperplasia, alveolar, bronchiolar and squamous metaplasia and epithelial neoplasia. The histological and the morphometric findings of the present study for the experimental group of the mice as compared to the control animals clearly demonstrate the lungs tissue damaging effects of the iron emission particles of the study area.

CONCLUSIONS

It has been demonstrated that the iron emission particulate matter in the atmosphere cause damage of uncertain degrees, to lungs particularly of the whetting workers. Thus human respiratory ailments of the whetting workers need prompt studies and remediation measures. Immediate response to the latter may include proper exhaust and mandatory use of respiratory filters for the workers.

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