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Evaluation of Toxic Potentiality of Carbon Black on Respiratory Tract of Rats

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Abstract

Introduction: *Particle pollution is a threat to human health, especially Nanoparticle pollution is threatening. In the present study we are studying the toxicity of CARBON BLACK to assess their potential in damaging human health. Since carbon is an inhalational component of our everyday lives.*

Method: *Rats are exposed to CARBON BLACK by inhalation and several parameters like haemoglobin%, RBC, WBC and Platelet Count, biochemical and hystopathological studies of exposed lung in comparison with blank.*

Results: *Lung weights of rats exposed to carbon black were a little higher than the control group due to accumulation of lung fluid. It also showed an increase in WBC, RBC and Platelet count. 21 days after inhalation an acute neutrophil influx into the airspaces, with accompanying increased epithelial permeability. The lungs of the rats exhibited histological changes consistent with an inflammatory response, particle retention, and penetration of particles into the deep lung at the highest doses.*

Conclusion: *The results obtained in the present study indicated that the carbon black particles of $PM_{2.5}$ was retained in regions of the lungs, suggesting possible physical interaction of particles with surrounding cells and tissue and causes potential lung injury.*

Keywords: ***Nanoparticles, Carbon Black, Rats, Haematology, Oxidative Stress.***

INTRODUCTION

That particle pollution is a threat to human health and has to be regulated. Pharmacists and combustion engineers normally define all particles below 1µm as nanoparticles. In toxicology and material sciences, the cut point is set to particles below 100 nm in one dimension. Exposure to nanoparticles will mainly be by inhalation, ingestion and less through the skin. Inhalation of particles is considered to be the most important and critical exposure route, because of the dustiness of the materials and the high deposition rate in the peripheral lung after inhalation.[7] Inhalation of particles can cause diverse types of lung reactions and lead to development of diseases.[15] The mechanisms behind the toxicological effects of particles are still being debated, but relevant parameters include inflammation and oxidative stress.[14]

MATERIALS AND METHODS

5-5'dithio bis-2-Nitro benzoic acid (DTNB) reagent, Trichloroacetic acid, Thiobarbituric acid, Glutathione, Phenol reagent (Folin and Ciocalteau's Reagent) were procured from Himedia Laboratories Ltd, Hyderabad. Bovine serum albumin was procured from Zeal Chemicals, Hanamkonda. 18 Adult healthy male Wistar rats weighing about (150±10g) were used for the study. They were caged and fed at standard conditions according to CPCSEA Guidelines. Rats were exposed to CB in the inhalation chamber at total concentrations of 200mg i.e,16.37mg/m^3 /each animal for 1hr/day, 5 days per week for a total of 4 weeks. The control rats were exposed to clean air containing no CB for the same period. Particle diameter of CB was measured by a particle size analyzer (Zeta potential) at (KELVN LABS, SURA LABS-Hyderabad).

PHYSICAL PARAMETERS

The body weight of each group were measured just before and 21 days after treatment. Lung weights of all rats are measured after post treatment sacrifice.

Haemogram: Blood parameters are measured after collection of blood. Parameters such as follows are detected.

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- Red blood cell count (RBCC)
- White blood cell count (WBCC)
- Haemoglobin Percentage (Hb %)
- Platelet Count

BIOCHEMICAL ESTIMATION

Isolation of Lung tissue

All the groups of rats after 21st day were euthanized using ketamine followed by cervical dislocation. One gm of lung tissue homogenized in 10 ml ice cold phosphate buffer. The prepared homogenates were centrifuged and used for the determination of antioxidant parameter. The lung tissue homogenate (10% w/v) was prepared with 0.1 M Tris- HCl buffer (pH 7.4). The tubes with homogenate were kept in ice water for 30 minutes and centrifuged at 4°C (2500 rpm, 10 min). The supernatant of homogenate thus obtained was separated, used to assay and estimate total protein content, malondialdehyde, reduced glutathione.

BRONCHOALVEOLAR LAVAGE

The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (60mg/kg).for each animal, the tracheal chain was prepared, tracheal cannuala was inserted and the lungs were lavaged with 5ml of saline 4 times. Cells were removed by centrifugation (1500 rpm for 10min) and the pellet was resuspended in 0.5 ml saline. 0.2 ml of geimsa stain in buffered saline (pH 6.5) was added to it. After 5 minute the number of each type of leukocytes in 0.5 ml fluids was determined under the microscope 450 X magnifications. Fluid from the first lavage was pooled for evaluation of biochemical changes after cell removal. The LDH level and total protein was determined.

HISTOPATHOLOGY OF LUNG

Samples of lung was stored in the fixative solution 10% formalin and the samples of sciatic nerve tissue were paraffin embedded, cut into thin sections with 4 µm thickness size. Staining was done by using hematoxylin and eosin. Lung sections were examined under light microscope (100x).

Transmission Electron Microscopy (TEM) ANALYSIS

Samples were fixed in 2.5%-3% glutarldehyde in 0.1M phosphate buffer (pH 7.2) for 24h at 4˚C, and washed with PBS for 3-4 times each 30-45 minutes, then post fixed in 2% aqueous Osmium Tetroxide for 2H later washed with deionized distilled water for 4-6 times each 30-45 minutes, dehydrated in series of graded alchohols, infiltrated and embedded in araldite 6005 resin or spur resin (spur 1969).Incubated at 60-80˚C for 48-72h for complete polymerization. Ultra thin (50 - 70 nm) sections were made with a glass knife on ultra microtome (Leica Ultra cut UCT-GA-D/E-1/00), mounted on copper grids and stained with saturated aqueous uranyl acetate (UA) and counter stained with Reynolds lead citrate (LC). Viewed under TEM (Model:Hitachi,H-7500 from JAPAN) at required magnifications as per the standard procedures at RUSKA Lab's college of Veterinary Sciences, SVVU, Rajendranagar, Hyderabad, India.

STATISTICAL ANALYSIS

All the results were expressed as mean ± standard deviation. The data from the physical, behavioral, hematological results were statistically analyzed by unpaired student's t-test by using Graph pad prism Version-6.0 software. The data from the biochemical results were statistically analyzed by unpaired student's t-test. The p-value <0.05 was considered to be statistically significant

RESULTS

Analysis of carbon black particle size:

Particle diameter of CARBON BLACK was measured by a particle size analyzer and the concentration of CARBON BLACK particles below 100nm was approximately 2.3% and 97.7% volume of the CARBON BLACK particles are having diameter at 213.5nm.

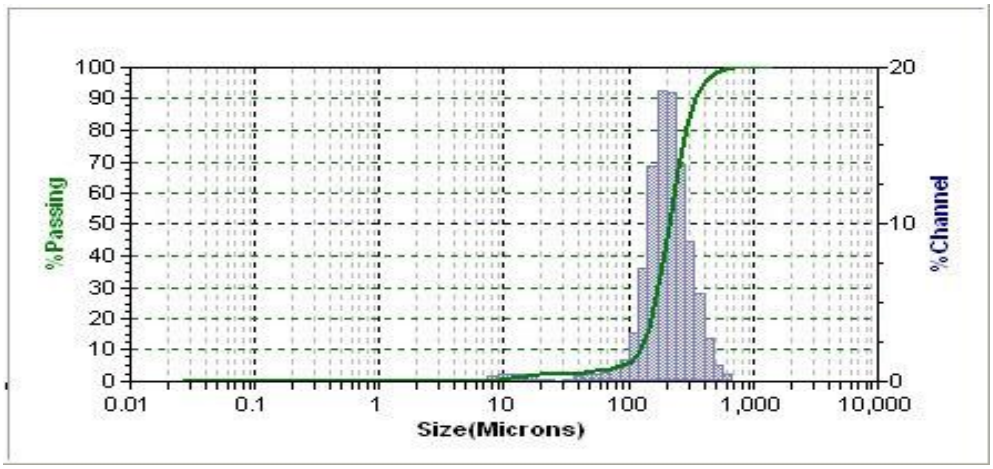

Figure 1: Particle Size Analysis Report.

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PHYSICAL EXAMINATION

LUNG WEIGHT

Significant increase in CARBON BLACK treated group was observed when compared with that of normal control.

Table 1: Effect of carbon black on lung weights in rats

Treatment	control	CARBON BLACK
Lung body weight	1.170 ± 0.0096	1.437 ± 0.018[a]

Values are expressed as Mean ± SD (n=6); P value is significantly different (compared with control using student's t-test) a -P < 0.0001 (compared with control using student's t-test).

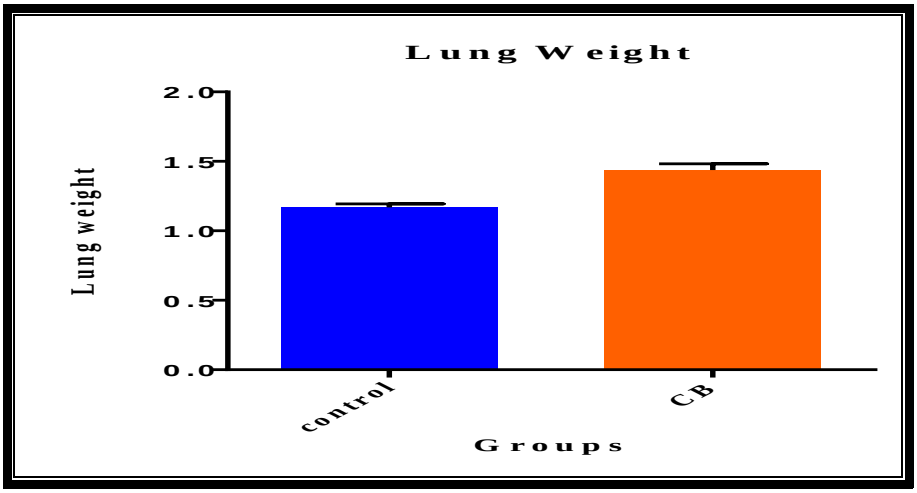

Figure 2: Effect of carbon black on lung weights

Haematological Estimation

Table 2: Effect of carbon black on hematological tests in rats

Groups	RBC(x10^6 cell/mm^3)	WBC(x10^3 cells/mm^3)	Haemoglobin (%)	Platelet count (x10^{5}cells/mm^3)
Control	6.897 ± 0.034	8.483 ± 0.16	14.07 ± 0.14	61.00 ± 0.57
CARBON BLACK	7.705 ± 0.030[$]	9.817 ± 0.18[$]	15.03 ± 0.19[*]	67.27.00 ± 0.6146[$]

Values are expressed as Mean ± SD (n=6);
$ -P < 0.0001 (compared with control using unpaired students t-test)
**-P<0.05 (compared with control using unpaired students t-test's).*

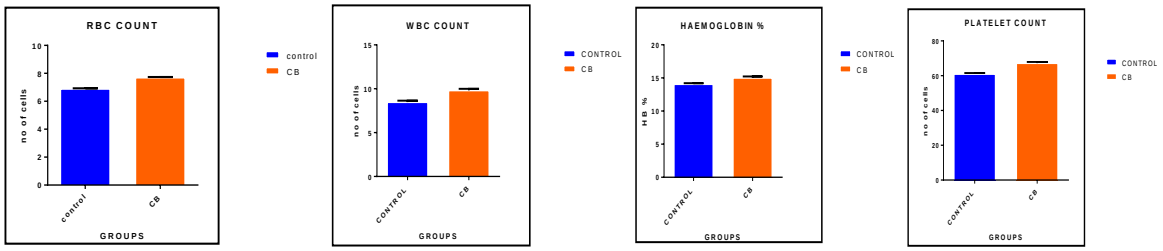

Fig. 3, 4, 5 & 6: Effect of carbon black on hematological tests in rats

BIOCHEMICAL ESTIMATION

Table 3: Effect of carbon black on biochemical tests in rats

Groups	Total Protein (mg/gm of tissue)	GSH (µg/mg of protein)	MDA (ng/mg protein)	LDH(u/l)
Control	5.756 ± 0.04702	62.78 ± 1.024	2.160 ± 0.05120	42.333±3.842
CARBON BLACK	9.853 ± 0.06013[$]	31.11 ± 1.111[$]	4.698 ± 0.07119[$]	99.166±7.890[$]

Values are expressed as Mean ± SD (n=6)
$-P < 0.0001 (compared with control using unpaired students t-test)

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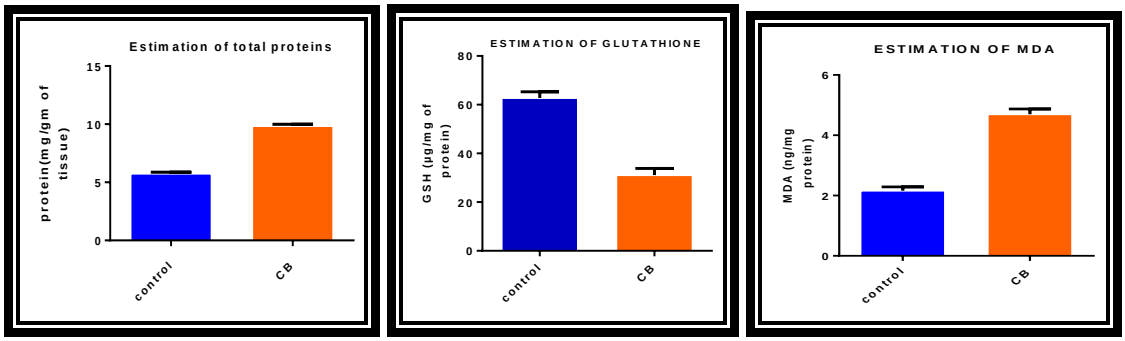

Fig. 7, 8 & 9: Estimation of total proteins, Glutathione and MDA in tissue homogenate.

BRONCHOALVEOLAR LAVAGE CELL DIFFERENTIALS

Table 4: BAL CELL DIFFERENTIALS

GROUPS	Total cell count	PMN	Monocyte	Lymphocyte	Eosnophill	Basophill
Control	2042±43.69	17.00±0.36	11.00±0.33	67.00 ± 0.36	4.000 ± 0.35	2.000 ± 0.31
Carbon black	3809±83.12$^{\$}$	38.00±0.34$^{\$}$	13.00±0.34$^{\$}$	36.00 ± 0.36$^{\$}$	9.000± 0.36$^{\$}$	3.833 ± 0.47$^{\$}$

Values are expressed as Mean ± SD (n=6)
$-P < 0.0001 (compared with control using unpaired students t-test)

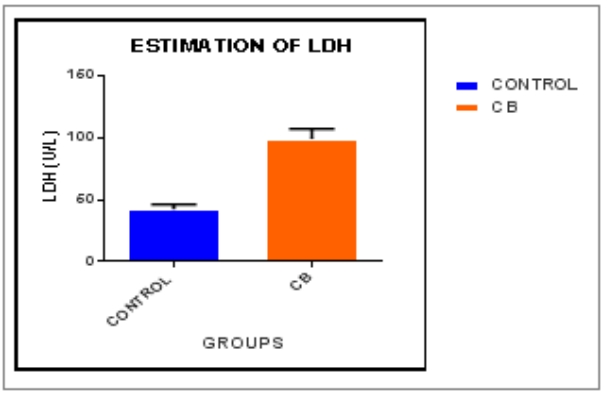

Fig. 10: Estimation of LDH level in BAL

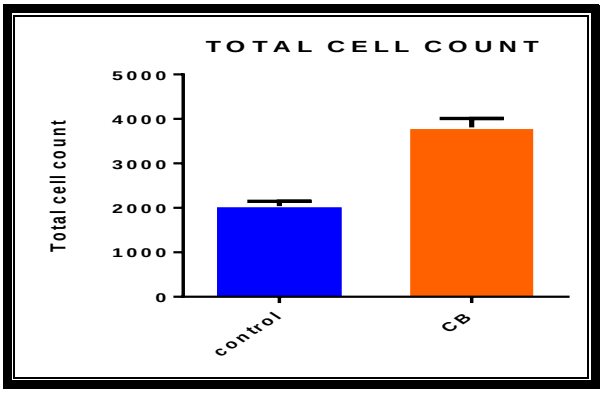

Fig.11: BAL Total Cell Count

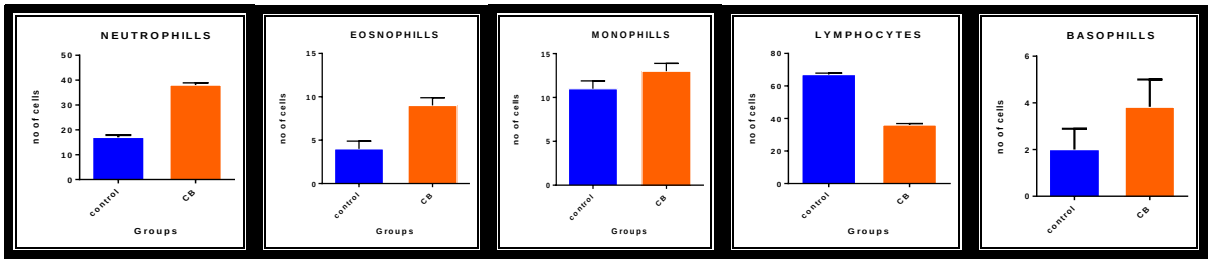

Fig. 12, 13, 14, 15 & 16: BAL CELL DIFFERENTIALS

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HISTOPATHOLOGY OF LUNG TISSUE

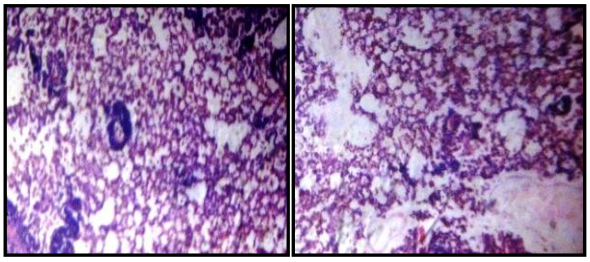

Fig.17 & 18: Control Tissue

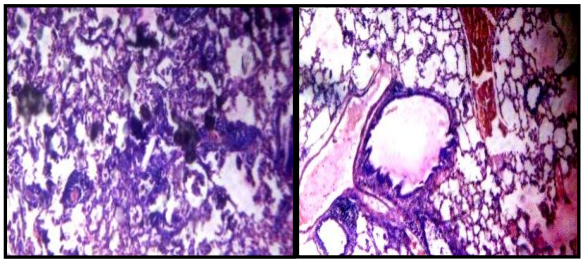

Fig.19&20: Carbon Black exposed Rat tissue

Histological analysis in rats exposed to **Fig. 19** carbon black when compared to **Fig. 17** control rats. Rats exposed to CARBON BLACK showed Infiltration of leukocytes (infiltration of eosinophils, neutrophils) when compared to control rats, status of bronchi (bronchoconstriction), perivascular status of lung blood vessels (infiltration of Mononuclear cells around the lung blood vessels). Integrity of Alveoli (alveolar emphysema, and hemorrhages).Histological analysis showed deposition of **Fig. 20** carbon black particles in rats exposed to carbon black when compared to **Fig. 18** control rats.

Histopathology of Lung by Transmission Electron Microscopy (TEM)

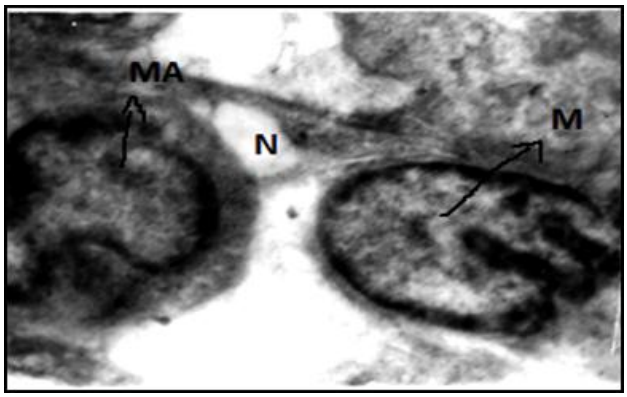

Figure 21: Section of the lung of a rat showing Mitochondria (M), normal nucleus (N), Macrophage (MA).

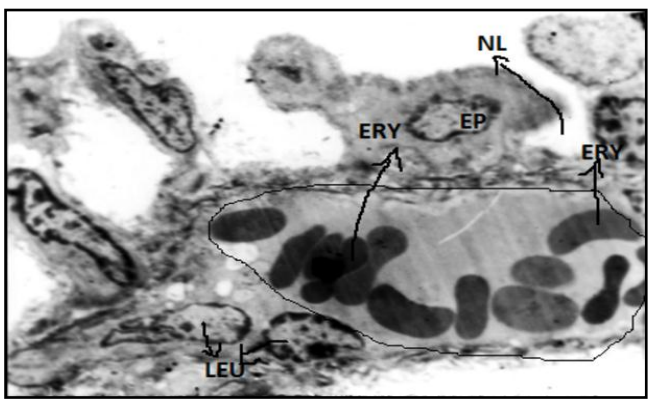

Figure 22: Section of the lung of a rat showing numerous Erythrocytes (ERY), narrow epithelial cell (EP), narrow lumen (NL), Leukocytes (Leu).

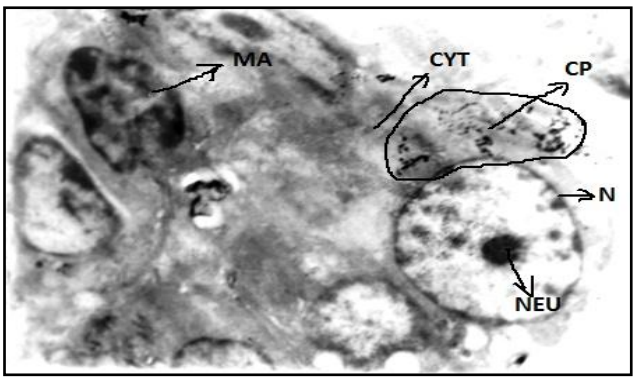

Figure 23: Section of the lung of a rat showing Nucleolus (NEU), Swollen Nucleus (N), Cytoplasm (CYT), Carbon particles (CP), Macrophages (MA).

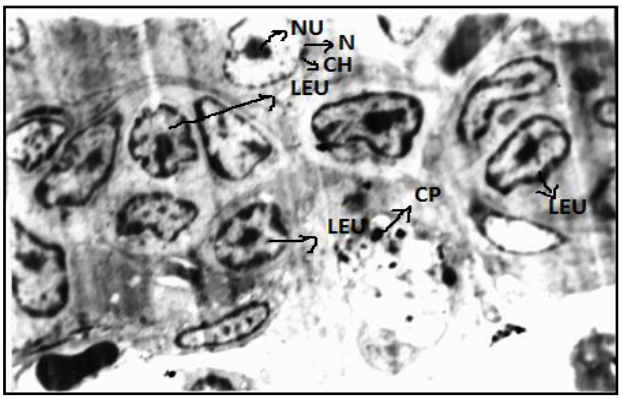

Figure 24: Cell showing numerous leukocytes (LEU), Chromatid material (CH), Nucleus (N), Nucleolus (NU), Carbon particles (CP).

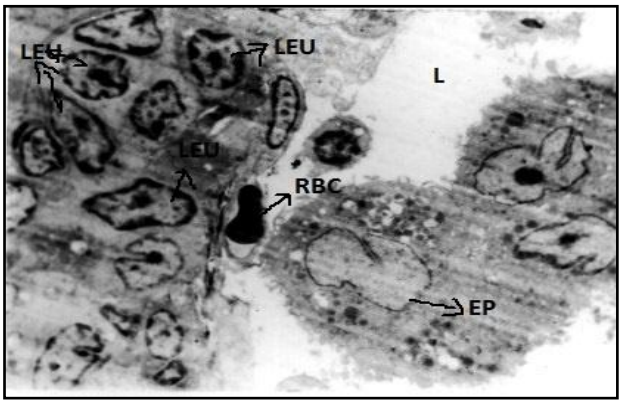

Figure 25: Section of lung of a rat showing red blood cells (RBC), Epithelial cell (EP), Lumen (L), Leukocytes (LEU).

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Histopathological examination of the lung tissues showed varied damage when compared with the control group. Lung tissues of Control rats showed normal nucleus, mitochondria, and epithelial cells **(Figure 21). (Figure 22)** shows numerous blood cells including erythrocytes, numerous mitochondria, and numerous leukocytes. Figure shows narrow alveolar lumen, narrow epithelial cells, and numerous leukocytes. **(Figure 23)** shows deposition of electron dense sphere carbon particles deposited in cytoplasm of epithelial cells. Swollen nucleus showing centrally placed nucleolus and nucleolus shows electron dense granular material. (**Figure 24, 25)** shows numerous mononuclear cells with different shape and size of chromatid material. One cell vesicle magnified for electron dense large bodies (carbon particles).

DISCUSSION

The present study is to evaluate toxic potentiality of carbon black on respiratory system in rats by assessing physical, hematological, apart from this biochemical and histopathological parameters in lung samples also revealed. Carbon black induced polycythemia, leukemia and thrombocytopenia in rats assessed by estimating the parameters- RBC, WBC, Haemoglobin percentage and platelet count. Inhalation of Carbon black of $PM_{2.5}$ caused neutrophil influx in rat lungs 21 days after inhalation, which accounted for increase of the total BRANCHO ALVEOLAR LAVAGE leukocyte numbers. An increase in WBCs count might indicate a disturbance in the function of immune system efficiency. LDH concentrations were increased in BRANCHO ALVEOLAR LAVAGE FLUID which causes epithelial injury. Carbon black induced oxidative stress (increased Protein, MDA levels and decreases glutathione) which was estimated in lung tissue homogenate. The results obtained in the present study indicated that the carbon black particles of $PM_{2.5}$ was retained in regions of the lungs, suggesting possible physical interaction of particles with surrounding cells and tissue and causes potential lung injury.

DECLARATIONS

Ethical Approval: The study protocol approved was approved byinstitutional animal ethical committee Talla Padmavathi College of Pharmacy, Warangal.

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