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# MgO Nanoparticle Synthesis, Chemical Delivery in Rat Model Induced Lung Injury

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## Abstract

Acute lung injury is a disorder of acute inflammation that causes disruption of the lung endothelial and epithelial barriers. Acute Respiratory Distress Syndrome or ARDS is a diffuse inflammatory lung process that frequently manifests in critically ill patients, with an estimated incidence of 3 million people a year. Clinically, ARDS is manifested by bilateral or diffuse radiographic infiltrates, hypoxemia, decreased lung compliance, and increased ventilatory dead space. The histological manifestation of ARDS is diffuse alveolar damage as defined by epithelial injury, hyaline membrane formation and alveolar flooding with proteinaceous fluid, increase alveolar surface area and frequently neutrophilic inflammation. The animal model correlate to ARDS as an acute lung injury (ALI). Models were employed to test potential new therapeutic interventions and to investigate under-lying mechanistic pathways that lead to diffuse lung injury. In this study cage cigarette smoke model was used for an acute lung injury. For that purpose, thirty (30) young albino healthy rats were used, and their duration of trial was 21 days. Each group was given an oral drug administration containing MgO-150mg/kg, MgO-300mg/kg BW for treatment and diet schedule in young albino rats of male sex for the experimental procedure of 0 to 21 days. Mean body weight, BALF, lipid profile, liver profile and lung histology were assessed. Results revealed that MgO nanoparticles exhibited antioxidant capacity at dosages of 300mg/kg, and 150mg/kg. This formulation of selected nanoparticles at high dose exhibited an acute lung injury and antioxidant effects as compared to low dose extract. Additionally, transcriptional factors revealed up-regulation at high dose in contrast to the low dose extract in lung injury treated groups.

**Keywords:** MgO nanoparticle, Body weight, Cigarette smoke, Transcriptional, Regulation.

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## INTRODUCTION

Acute lung injury is a disorder of acute inflammation that causes disruption of the lung endothelial and epithelial barriers. The alveolar–capillary membrane is comprised of the microvascular endothelium, interstitial, and alveolar epithelium. Cellular characteristics of ALI include loss of alveolar–capillary membrane integrity, excessive transepithelial neutrophil migration, and release of pro-inflammatory, cytotoxic mediators. Biomarkers found on the epithelium and endothelium and that are involved in the inflammatory and coagulation cascades predict morbidity and mortality (Matthay and Zimmerman 2005). Lung injury and the acute respiratory distress syndrome describe clinical syndromes of acute respiratory failure with substantial morbidity and mortality. Even in patients who survive acute lung injury, there is evidence that their long-term quality of life is adversely affected (Sasannejad, Ely & Lahiri, 2019). The American/European Consensus Conference defined patients as having lung injury and the acute respiratory distress according to the ratio of partial pressure of oxygen in arterial blood ($PaO_2$) to the inspired fraction of oxygen ($FiO_2$) being less than 300 (ALI) or less than 200 (ARDS) (Stine & Lewis 2016).

Clinical acute lung injury is associated with specific risk factors that can be broadly divided into intra-pulmonary conditions, including pneumonia, aspiration, and blunt trauma; and extra-pulmonary risk factors, including extra-pulmonary sepsis, trauma, significant blood product resuscitation, and pancreatitis. Several clinical disorders can initiate ARDS, including pneumonia, sepsis, gastric aspiration, and trauma (Altemeier, Hung & Matute-Bello, 2017). The histological manifestation of ARDS is diffuse alveolar damage as defined by epithelial injury, hyaline membrane formation and alveolar flooding with proteinaceous fluid, formation of microthrombi and frequently neutrophilic inflammation (Albaiceta *et al*., 2021). New potential concerns for occupational and environmental lung disorders are posed by nanomaterial. Certain nanoparticles, namely nanosized metals (such as nickel, and cobalt), should be properly identified as risk factors for lung disorders due to their numerous impacts in humans. Other nanomaterials, like fullerenes and carbon nanotubes, have been shown to cause inflammatory, immunological, and/or fibro genic effects in mice and rats (Bonner *et al.*, 2010). MgO is also used as detoxifier, and for bone regeneration in addition to relieving heartburn, stomach pain, and acid reflux. Magnesium oxide in vivo toxicity studies have not yet been reported. Therefore, it may be interesting to assess the pulmonary toxicity of MgO nanoparticle in rats. This investigation investigated the pulmonary toxicity of MgO nanoparticle in rats (Gelli *et al*., 2015).

There are many potential paths in bio-nanotechnology that could be used to treat lung injuries. Numerous nanoparticles, such as zinc oxide, casein, and different 0 polymers, have been studied for their potential to prevent lung injury. The design and testing of many nanoparticles for oral delivery of treatment for lung injury is now occurring (Donaldson *et al*., 2006). Clinically, ARDS is manifested by bilateral or diffuse radiographic infiltrates, hypoxemia, decreased lung compliance, and increased ventilatory dead space (Dreher *et al.*, 2020). At the same time, NPs exhibit unusual adsorptive properties and fast diffusivities and they are not stable in critical conditions (Bindhu *et al*., 2016). Metal oxide NPs (MONPs) possess some advantages such as high stability, simple preparation processes, easy engineering to the desired size, shape and porosity, no swelling variations, easy incorporation into hydrophobic and hydrophilic systems and easy functionalization by various molecules due to the negative charge of the surface, that make them a promising tool for biomedical applications (Rafiei-Sarmazdeh *et al*., 2019).

Recently studies, aimed at understanding the toxicity mechanism of MgO NPs, indicated that physical injury occurred to the cell membranes, along with decreased motility and biofilm formation ability of R. solanacearum, due to the direct attachment of MgO NPs to the surfaces of the bacterial cells, which was observed by scanning electron microscopy (SEM) and transmission electron microscopy (Cai *et al.*, 2018). MgO is also used for the relief of heartburn, sore stomach, and acid indigestion, as an antacid, detoxifying agent, and for bone regeneration. Till date there is no report on in vivo toxicity studies of magnesium oxide. Hence, the evaluation of pulmonary toxicity of MgO NPs in rats is potential interest. The purpose of this study is to explore the pulmonary toxicity of MgO NPs in rats (Mangalampalli *et al.*, 2018). MgO exposure showed the dose-dependent infiltration of interstitial lymphocytes, lymphoid aggregation, dilated congested vessels, peribronchiolar lymphocytic infiltration, granulomatous reactions, and alveolar macrophages at 1 day post-exposure period and were worsened at 1 week period and were reduced and cured at 1 month post-instillation period, supporting the lung damage, which was comparable to Quartz NPs. From the above results, it was suggested that intratracheal instillation of these MgO NPs produced a significant increase in pulmonary enzymatic levels, which was supported by histopathological examinations, indicating the pulmonary toxicity of MgO NPs. This study also reported in vitro cytotoxicity of MgO NPs in human astrocytoma U87 (astrocytes-like) cells and in normal human fibroblasts (Gelli *et al*., 2015).

The pulmonary toxicity of MgO NPs in rats was may be due to the generation of ROS. Exposure of MgO NPs to rats can cause depletion or inhibition of the antioxidant systems and increasing the formation of ROS through mitochondria dysfunction. There are many evidences showing that NPs increase ROS production and can cause cell death in different types of cultured cells. Our previous reports showed that the elevated levels of MDA and depletion of glutathione, Catalase, SOD, and TAS activity are strongly correlated to ROS generation and lipid peroxidation, indicating the induction of marked oxidative stress in rats (Kim *et al*., 2009). Neutrophils are shown adhering to the injured capillary endothelium and margination through the interstitial into the air space, which is filled with protein-rich edema fluid. In the air space, an alveolar macrophage is secreting cytokines, interleukin (IL)-1, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α, which act locally to stimulate chemotaxis and activate neutrophils. Interleukin-1 can also stimulate the production of extracellular matrix by fibroblasts. Neutrophils can release oxidants, proteases, leukotrienes, and other proinflammatory molecules, such as platelet-activating factor (PAF) (Zemans *et al*., 2019).

## MATERIAL AND METHODS

### Chemicals

Magnesium oxide nanoparticle (MgONPs), Cigarettes, smoke generating chamber, Trizol, ethanol, methanol, chloroform, Isopropanol, Nuclease free water, and de-ionized water.

**Place of Work**

The area of work and research was Government College University Faisalabad and Health Biology Lab department of Physiology.

**Experimental Design and Grouping of Animals**

The thirty (30) young albino rats with the weight of 150-300 grams was taken for research purpose from the animal station, Physiology department, GCUF. The male young albino rats was subjected to acclimatization at suitable environmental conditions of animal house temperature, and surrounding humidity (40 to 60%) for 21 days, routine diet, water intake and medicine was available to animals, some ethical condition are includes, on caring and use of animal model for the prevention of severe health related problems were taken on bio ethics committee Government College University Faisalabad, Pakistan was followed. Thirty (30) male young albino healthy rats were used and their duration of trial was 21 days. The first group was kept as a Negative control group. The second group was kept as a positive group. The group number three and four were taken MgO nanoparticle administration via oral gavage. The given dosages were 150mg/kg, 300mg/kg respectively. The doses were synthesized by addition of nanoparticles in distilled water. The group number five was kept as a standard group. The rats were kept in well-aerated cages with free access to a chow maintenance diet (CMD) and water at a temperature of 25±2 ºC. These dosages were labelled as low dose group (LDG=150mg/kg body weight), high dose group (HDG= 300mg/kg body weight).

**Diet for Animals**

A 100 g of CMD consisted of 69.36% corn starch (69.36g), 12% protein (12g), 4% corn oil (4g fat), 4% minerals (4g), 4% cellulose (4g), 1% vitamin mixture (1g vitamins), 0.3% methionine (0.3g), and 0.2% choline chloride (0.2g). Diet was kept in a dark dry area.

**Establishment of CS-Induced Lung Injury Model**

Animals were randomly divided into five groups. All groups were identified by their tail. Each group of rats 'tail was marked by different marker colors. Fresh air was available for Negative Control group and four groups were affected by Cigarette Smoke. Cigarette smoke exposure (CS) generated by Gold Flake Cigarettes, Company, Pakistan Tobacco Co. Ltd. (nicotine: 6.17 mg). In the CS- induced lung injuries model group, the rats were completely exposed to smoke during trial on daily basis, four cigarettes per day. During this period, the behavior, morphology, and fatality rate were considered. In addition, lung injury was evaluated by BALF test.

**Cigarette Smoke Exposure System**

The system used to expose rats to side stream cigarette smoke consisted of a peristaltic pump, a smoke-generating chamber, and a whole-body CSE chamber connected in a series by silicone tubes. The ventilator pump was set to deliver 150 mL of air every 10s. The smoke-generating chamber consisted of an acrylic cylinder (height, 27 cm; diameter, 16 cm) corresponding to a total volume of 5430 $cm^3$ in which one cigarette was constantly lit at a time. Then smoke was delivered to an inhalation chamber (length, 40 cm; width, 20 cm; height, 25 cm) of 20,000 $cm^3$ total volume and exhausted through a hole. All rats were exposed at a time into the smoke chamber for 30 min. All rats marked with different marker colors for identification of their groups (Dianat *et al*., 2018).

**Sample Collection**

After twenty-one days of treatment; samples were collected. At the end of our experimental trials, the animals were euthanized and organ and tissue samples were collected (i.e. Lungs) and preserved in neutral buffered formalin for histopathology. Blood samples were taken and centrifuge at 4000rpm for 8-15 minutes to separate plasma and serum. Further, separated serum was stored at -20$^{O}$C for analysis. Rats were disposed in biodegradable bags.

**Evaluation of Lung Injured Rats**

**Bronchoalveolar Lavage Fluid (BALF)**

An established method for examining the cellular components of the airways and air gaps is bronchoalveolar lavage (BAL). A catheter is inserted into the rat's trachea and a saline solution is infused into the bronchioles before the infused fluid is slowly pulled to isolate BAL fluid (Dianat *et al.*, 2018).

**The Procedure of BALF**

Rats were initially cut into the neck region for exposing the trachea and lungs by slicing through surrounding tissues. A needle gauge with plastic tubing was then placed into the lungs after the tracheal area was bolded using forceps. One milliliter of normal saline was withdrawn through the needle gauge, and the rat's lungs were then filled with the normal saline. The fluid was retained in the lung for an additional 30 to 60 seconds after the instillation process, which took almost 30 seconds. The liquid was then carefully aspirated back into the chamber and collected into Eppendorf's.

After that, a temperature-controlled centrifuge was used to centrifuge the collected BALF at 1000 X for 10 minutes at 4°C. The supernatant was then transferred to a fresh set of Eppendorf tubes, which were promptly frozen at -40 °C in preparation for other tests such the TAC, TOS, and MDA assays.

**Total Antioxidants Capacity (TAC; mmol Trolox equivalent / L); Total Oxidant Status (TOS; µmol $H_2O_2$ Equiv. $L^{-1}$)**

For TAC, a biochemistry analyzer (Biosystem BTS-330) to examine the antioxidant capacity of the serum sample. A unicolor wavelength of 660nm was designated for this protocol. The first reading was done by using blank (water). A 12.5µl serum sample was

mixed with 600 µl of acetate buffer and then 50 µl ABTS added to this solution. The solution was incubated for 4-5 min at 37ºC then 2nd reading of absorbance was taken. By the difference between the 2nd and 1st reading, delta absorbance was obtained. The final concentration was calculated by the calibration. For TOS; a total of 70µl of serum sample was added to a test tube. A 450 µl of reagent 1 was added to the same test tube. Mixture were mixed gently. Reagent 2 with a quantity of 22 µl was added and mixed well. Incubation was carried out of these contents for 5 min at 37°C in the absence of light. Absorbance was taken at a wavelength of 545nm to quantify the TAC in the serum sample (Anwar *et al*., 2007).

**Malondialdehyde (MDA; nmol/ml)**

Malondialdehyde (MDA) serves as the primary indicator of lipid peroxidation since it is the principal byproduct of processes involving the oxidation of fatty acids. Thiobarbituric acid reactive substances (TBARS) generated in the lung are typically used to measure the degree of lipid peroxidation. Malondialdehyde (MDA) level was determined by the serum sample spectrophotometrically. Serum sample absorbance was recorded at a wavelength of 532nm.

**Malondialdehyde Protocol**

First, label two equal sets of Eppendorf tubes according to the number of samples. Add the subsequent elements, one by one, to each of the Eppendorf tubes: Sample (25 µl). SDS (25 µl) from prepared stock solution of 8.1 %. Acetic acid (190 µI) from made stock solution of 20% Thiobarbituric acid or TBA (190 µl) from prepared stock solution of 0.8 %. Distilled water (75 µ1). After adding the above-mentioned components, all the Eppendorf tubes were kept in a water bath at 95 degrees centigrade for 1 hour for incubation. After 1 hour of incubation, all the Eppendorf tubes were cooled down at room temperature, after that 625 µl of Butanol (from prepared Butanol & Pyridine solution) was then added. Next, all of the Eppendorf tubes were given a vigorous shake before being centrifuged for approximately 10 minutes at 4000 RPM. The supernatant was then taken out in a new set of Eppendorf's tubes and then absorbance of the supernatant was then taken at 532 nm.

**Liver Function Analysis**

Serum Aspartate transaminase (AST) concentration was determined by the commercially available (Bio-active KIT LOT BD80932) kit method. It measures each sample's Wavelength at 546nm in comparison to the reagent blank. Serum ALT (Alanine transaminase) concentration was determined by the commercially available (Bio-active KIT LOT BD 80922) kit method. Measure each sample's Wavelength at 340nm in comparison to the reagent blank. Water absorbance 0.1240.

**Histopathological Examination:** It was conducted by the routine protocol for lungs tissue.

**Statistical Analysis**

One-way analysis of variance (ANOVA) followed by LSD tests were used to compare the means among the studied groups. The data obtained in the present study were expressed as mean ± SD for quantitative variables and statistically analyzed by using SPSS program (version 17 for windows) (SPSS Inc. Chicago, IL, USA). P value <0.05 was considered statistically significant. However, results of all parameters were compared using two-way ANOVA at GraphPad Prism 8.0 and Co-Stat computer software.

## RESULTS & DISCUSSIONS

The analysis of the data consisted on two phases. In first phase, characterization, Zetasizer and Ultraviolet Visible Spectroscopic Analysis. In second phase, the data was the data was analyzed statistically in tabulation form represented by descriptive.

**Mgo Nanoparticle Characterization**

**Zetasizer Analysis of Magnesium Oxide (MgO) Nanoparticles**

The Zetasizer was employed for the evaluation of exact size Zetasizer Nano ZS90 Malvern Panalytical Ltd). The average size of MgO nanoparticles ranged between 25 to 35nm (Figure.1).

**Ultraviolet Visible Spectroscopic Analysis of Magnesium Oxide (MgO) Nanoparticles**

A detailed Spectroscopic analysis of MgO nanoparticles was perfumed. The spectrophotometry (Perkin-Elmer-40 spectrophotometer); exhibited a peak at 295 nm. This is representing of Magnesium oxide nanoparticles (Figure.2). The finding was in agreement with another study (Somanathon *et al*., 2016).

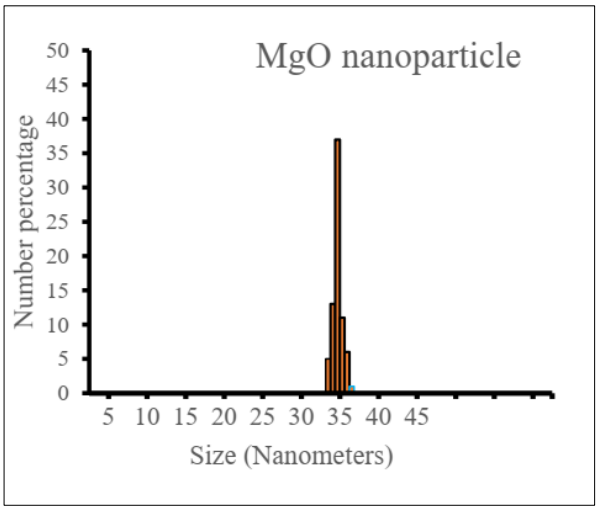



**Figure 1**

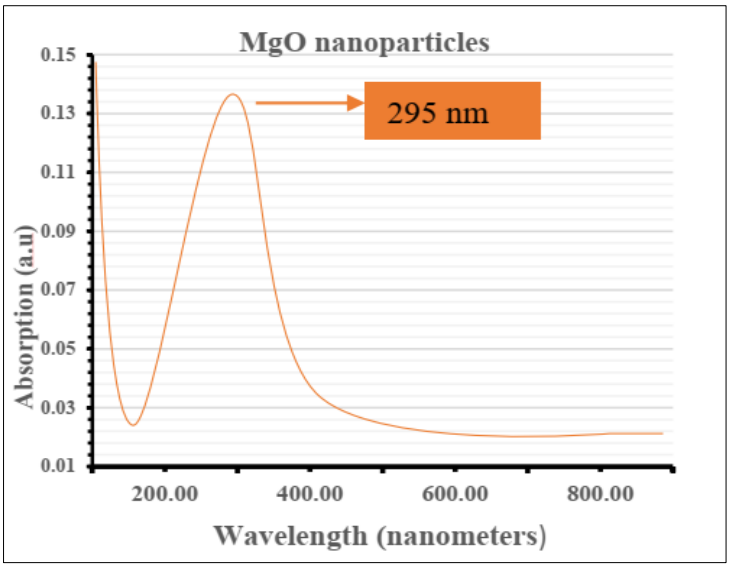



**Figure 2**

**Mean Body Weight Analysis**

The increase or decrease in mean body weight of each group was evaluated i.e. at day 7, day 14 and day 21 using two-way analysis of Variance and it presented a significant difference (figure 3; P≤0.05). A significant increase in treatment groups, i.e., LDG, HDG after the 2nd week of the trial was observed. According to this result, the positive control group revealed a statistically significant decreased in mean body weight (P<0.05). The Negative Control Group (NCG) indicate an increase in the mean body weight (P<0.05). A significant increase in mean body weight was recorded in the MgO-150 and MgO-300 and groups following a phase of initial decreased then it was increase at day 14, day 21 (P<0.05).

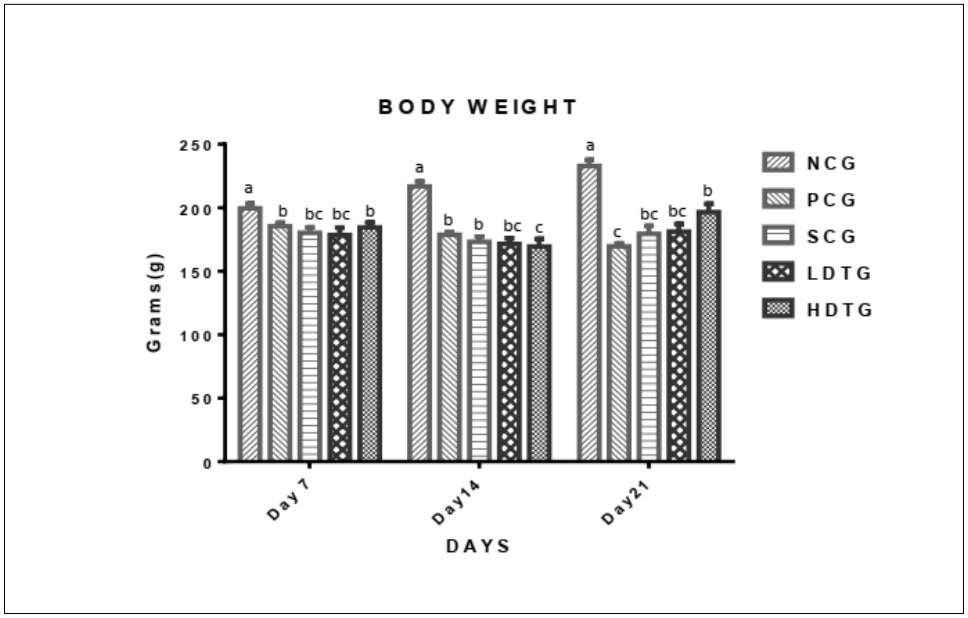



**Figure 3: Effect of MgO nanoparticle on Body weights of cigarette smoke induced acute lung injury-rat model**

Results are mean body weight (grams ±SE) of the Negative Control group, (NCG), Positive control group (PCG), Standard Control Group (SCG), Low Dose Treatment Group (LDTG), High Dose Treatment Group (HDTG), MgO low dose at 150mg/kg and high dose at 300 mg/kg on different days i.e. day7, day14, day21. Means values having different superscripts are significantly different from each other (P≤0.05).

**Bronchoalveolar Lavage Fluid Analysis (BALF)**

The Bronchoalveolar Lavage Fluid Analysis (BALF) evaluated among different groups at day 14. The positive control group showed a statistically significantly increased the alveolar destruction and surface area (P<0.05). The Standard Control Group (SCG) exhibited an increase the alveolar destruction and become maximally normalized (P<0.05). The MgO-300mg/kg

determined the quantifiable results as compared to low dose MgO-150mg/kg (Figure.4).

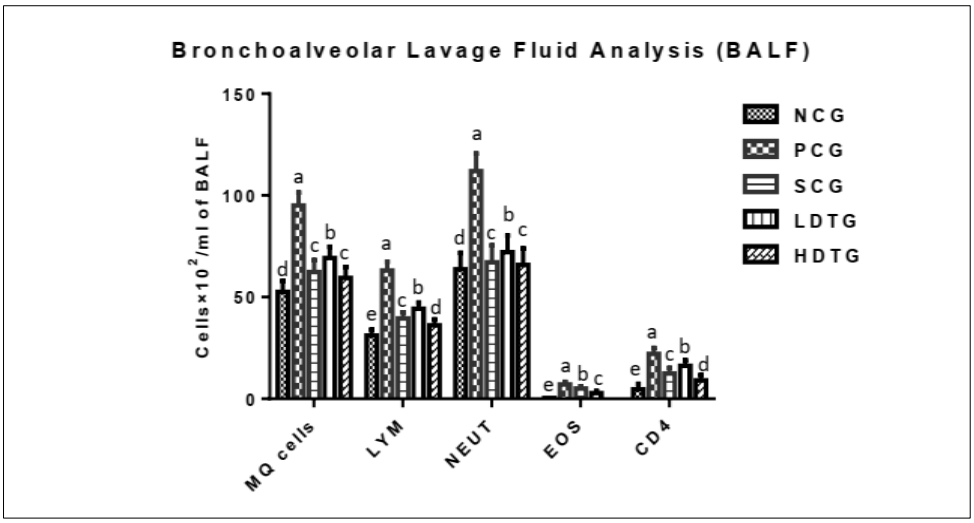
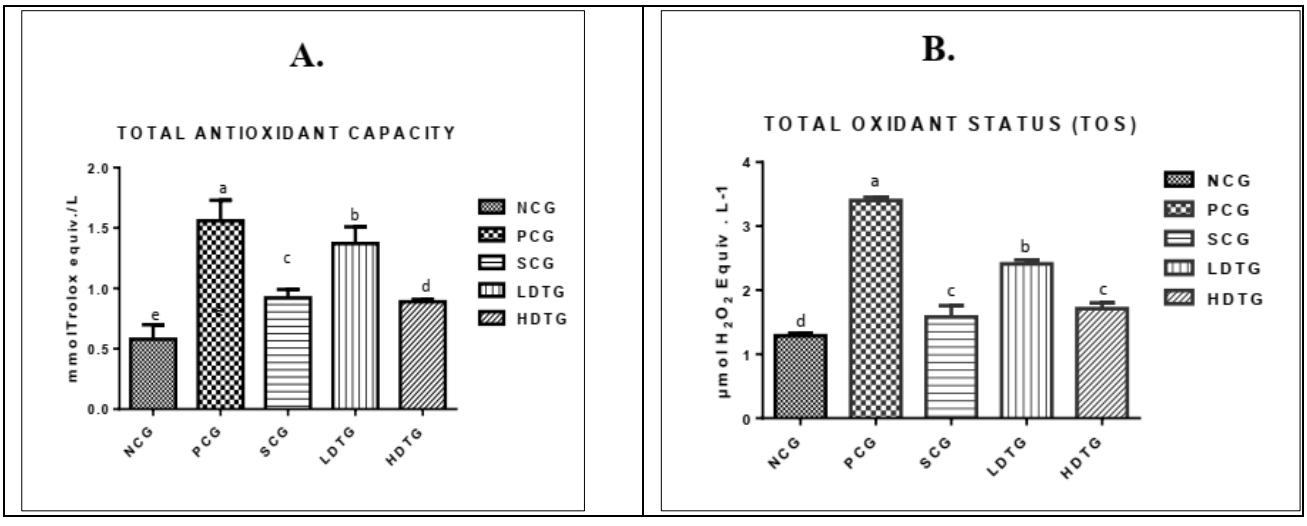



**Figure: 4 Effect of MgO nanoparticles on serum concentration of Bronchoalveolar Lavage Fluid (cells×$10^2$/ml).**

Different cells count of Bronchoalveolar Lavage Fluid (BALF) in Negative Control Group (NCG), Positive Control Group (PCG), Standard Control Group (SCG), Low Dose Treatment Group (LDTG), (MgO-150), High Dose Treatment Group (HDTG), (MgO-300).

**Total Antioxidant Capacity, Total Oxidant Status, Malondialdehyde Assay**

Total antioxidant capacity analysis the positive control group having a highly significant difference from the negative control group i.e., 1.56±0.17 mmol Trolox equivalent/L as compared to 0.58±0.12. ANOVA was used for statistical assessment of serum total antioxidant capacity (TAC) which declared significant difference among treatment groups (P<0.05). Mean serum TAC level at MgO-300 group were represent significant values to decreased TAC level than MgO-150mg/kg i.e., 1.37±0.14, 0.89±0.02 as opposed to 0.58±0.12 (Figure5A; P<0.05). One-way ANOVA was used to determine the serum TOS level in each group. It is directly corresponded with increased total oxidant status (TOS). In positive control group the values of TOS significantly increased (P<0.05). Statistically considerable difference showed in positive control and Negative control group 3.40±0.05 $H_2O_2$ Equiv. $L^{-1}$, as compared to 1.29±0.04· Standard Control group significantly decreased but MgO-300mg/kg revealed a significant decreased in the TOS than MgO-150mg/kg 2.41±0.06, 1.71±0.09 as opposed to NCG 1.29±0.04 (Figure 5B; P<0.05). ANOVA was applied for the overall analysis of serum MDA levels among different groups. It indicated a decrease in the HD group i.e., 3.8±0.07 as opposed to PC 5.4±0.11 (figure 5C; P≤0.05).



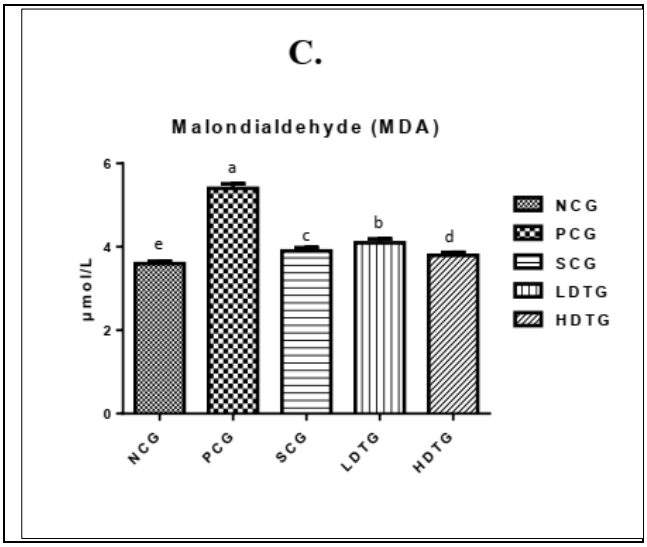



**Figure 5: Effect of MgO nanoparticle on serum concentration of TAC (5A; mmol Trolox equivalent/L), TOS (5B; µmol $H_2O_2$ Equiv.$L^{-1}$); MDA (5C; nmol/L)**

Results are means ± SE. NCG (negative control group), PCG (positive control group), SCG (standard control group), LDG (low dose group), and HDG (high dose group) in an acute lung injury rat-model. Means values having different superscripts are significantly different from each other (P≤0.05).

**Aspartate Aminotransferase (AST; U/L) and Alanine Transaminase (ALT; U/L)**

One-Way ANOVA was used for serum aspartate aminotransferase (AST) test, each group has quantifiable effect at the Standard Control Group (SCG). Test was performed by Bio-active kit AST KITLOTBD80932 (U/L). The aspartate aminotransferase was found to be significantly increased in PCG as compared to NCG and HD groups i.e., 65.28±2.1 as opposed to 44.5±2.6 and 47.1±1.9 respectively ($p<0.05$). It was observed that the mean concentration of AST in treatment groups reduced (Figure 6A; $P<0.05$). It was noted statistically significant increase in positive control group. Test was performed by Bio-active kit AST KITLOTBD80922 (U/L). This clarified that acute lung injury also effect on liver health. In MgO high dose 300mg/kg and low dose MgO-150mg/kg was significant decreased the level of ALT rather than to the positive control groups i.e., 26.41±1.59, 29.01±1.54, as compared to 36.30±1.71 (Figure 6B; $P<0.05$).

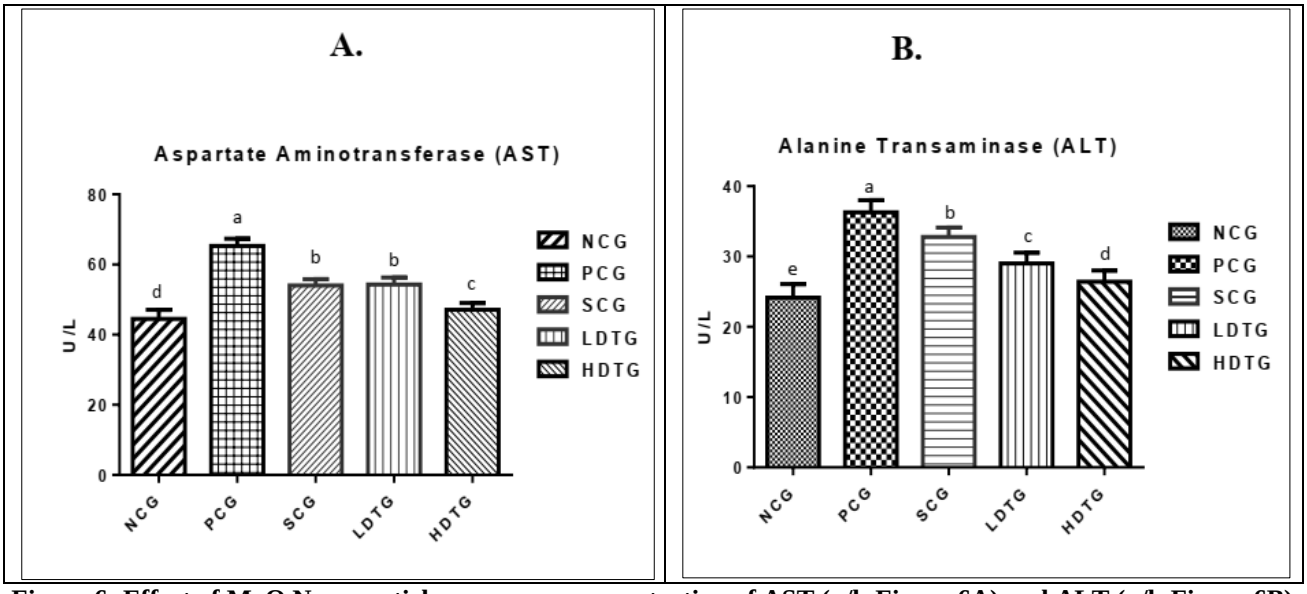



**Figure 6: Effect of MgO Nanoparticles on serum concentration of AST (µ/l; Figure 6A) and ALT (u/l; Figure 6B)**

Results are means ± SE. NCG (negative control group), PCG (positive control group), SCG (standard control group), LDG (low dose group), and HDG (high dose group) in an acute lung injury rat-model. Means values having different superscripts are significantly different from each other (P≤0.05).

**Lipid Profile**

**Total Cholesterol (mg/dl), Serum Triglyceride (mg/dl), Serum Low Density Lipoprotein (LDL), Serum High Density Lipoprotein (HDL).**

Mean serum total cholesterol level analysis evaluated a statistically significant difference among the various groups. Test was performed by Kit (IVD REF 230004). The standard control showed a statistically significant decreased in the serum total cholesterol level. The level of total cholesterol (TC) was significantly decreased in treatment groups as compared to the PCG (figure 7; P≤0.05). Mean serum TC level was decreased in groups treated with MgO NPs LD, HD treated groups as compared to the PCG, i.e., 135.14±5.89, 108.13±4.32 as opposed to 158.45±6.56 (Figure 7A; P≤0.05). Mean Serum Triglyceride level analysis evaluated a statistically significant difference among the various groups. Bio-active kit was used KIT LOT BD601032 to analyze the serum triglyceride level. The level of TG was significantly increased in PCG as compared to NC group i.e., 134.45±1.76 as opposed to 90.6±1.82 (P≤0.05). Treatment groups low dose and high dose treated groups showed significantly result as compared to PCG i.e., 126.09±1.93, 95.42±1.79 as compared to 134.45±1.76 (Figure 7B; P<0.05). ANOVA analysis of LDL-Cholesterol levels among different groups displayed a significant decrease in treatment groups as compared to the PC group, i.e., 66.14±1.96 and 56.94±1.86 for HDG and LDG as opposed to 78.50±2.46 for PC group (Figure 7C; P≤0.05). The values of concentrations of HDL-Cholesterol were significantly decreased in treatment groups as compared to the PC group i.e., 76.14±4.91 and 58.10±460 for HDG and LDG as opposed to 86.45±5.21(Figure 7D; P≤0.05). The mean serum concentration of the standard control group was significantly decreased as compared to the positive control (Figure 7; P≤0.05).

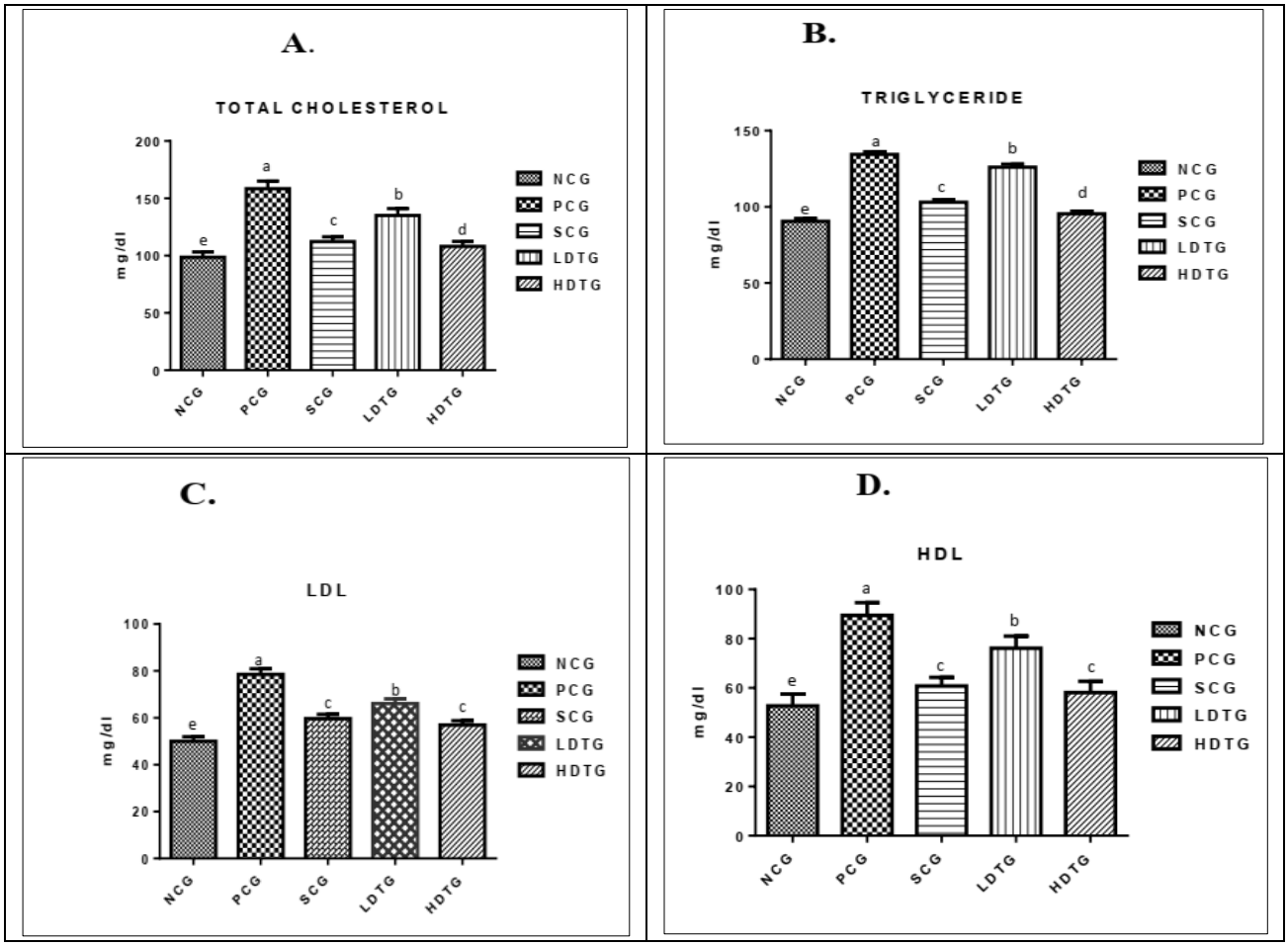



**Figure 7: Effect of MgO NPs on serum concentration of TC (7A; mg/dl), TG (7B; mg/dl), LDL (7.C), HDL (7.D)**

Results are means ± SE. NCG (negative control group), PCG (positive control group), SCG (standard control group), LDG (low dose group), and HDG (high dose group) in an acute lung injury rat-model. Means values having different superscripts are significantly different from each other (P≤0.05).

**Hematological Parameters**

**Complete Blood Count (CBC)**

Complete Blood Count (CBC) in this study we were determined different parameters such as Hemoglobin, Platelets, Red Blood Cell, White Blood Cells etc.

**Hematological indication in Red Blood Cells (RBC=x10$^6$/cubic millimeter), Hemoglobin (g/dl), White Blood Cells (WBC=x10$^3$/ mm$^3$) (µL), platelets (µL).**

One-Way analysis of variance was used to determine the serum red blood cells (RBCs) in different groups. Erythrocytes were significantly decreased in positive control group as compared to Negative Control group in Cigarette smoke induced an acute lung injury i.e.,6.45±0.59 as opposed to 7.75±0.85 (P<0.05). The level of serum RBCs was significantly increased in treatment groups, SCG, LDG, HDG as compared to PCG i.e., 7.28±0.64, 7.12±0.69 and 7.58±0.80 as opposed to 6.45±0.59 (Figure 8A; P≤0.05). Graph demonstrated that Hemoglobin were significantly decreased in positive control group as compared to NCG i.e., 9.5±0.9 as compared to 14.68±0.6 (P<0.05). MgO-300mg/kg showed better results, increased the level of hemoglobin as compared to low dose MgO-150mg/kg i.e., 14.17±0.59 as opposed to 12.9±0.48 (Figure 8B; P≤0.05).

White blood cells (WBCs) level were compared with different groups by using ANOVA. The serum level of WBCs was significantly decreased in PC group as compared to NCG i.e., 6.36±0.49 as compared to 8.19±0.60 (P≤0.05). MgO-300mg/kg revealed significantly increased serum WBCs level as opposed to PC group i.e., 7.92±0.60 as compared to 6.36±0.49 (Figure 8C; P≤0.05). Graph demonstrated that platelets count (PLT) were significantly decreased in positive control group as compared to Negative Control group (P<0.05) in Cigarette smoke induced an acute lung injury i.e., 428.32±30.5 as compared to 978.24±38.91. The level of serum RBCs was significantly increased in treatment groups, SCG, LDG, HDG as compared to PCG i.e., 849.06±33.8, 610.10±36.81 and 880.80±39.91 as opposed to 428.32±30.5 (Figure 8D; P≤0.05).

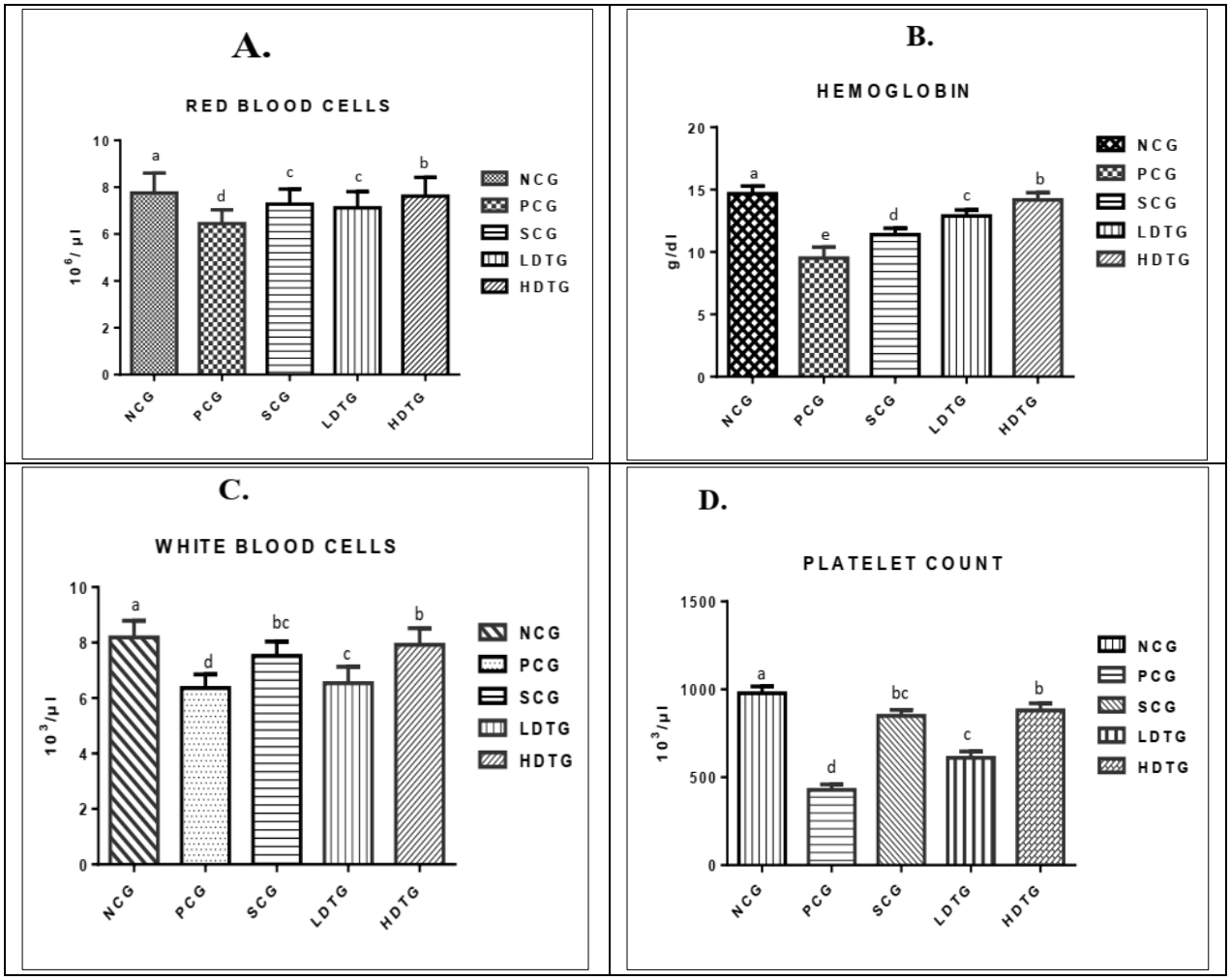



**Figure 8: Represented the Effect of MgO nanoparticle on serum concentration of Red blood cells (µl; 8A), Hemoglobin (g/dl; 8B), white blood cells (µl; 8C), platelets count (µl; 8D)**

Results are means ± SE. NCG (negative control group), PCG (positive control group), SCG (standard control group), LDG (low dose group), and HDG (high dose group) in an acute lung injury rat-model. Means values having different superscripts are significantly different from each other (P≤0.05).

**Histopathological Examination**

The MgO nanoparticle supplementation in Cigarette Smoke induced an acute lung injury rat model. Lung histology gave out a qualitative difference among different groups. A significant difference was noticeable between negative control and positive control. Group A, the negative control indicated normal architecture of the lung histology (Figure: 9A). The positive control showed a noteworthy change along with structural derangement in the tissue. Lung histology was seen in dark pink color (H&E; Magnification at 10X; figure: 9B). The positive control group displayed some morphological changes in lung tissues. PCG revealed destruction of lung tissues and large surface area in alveolar walls. Some tissues were irregular and shrunken (H&E. Magnification at 10X; figure: 9B). The standard control group showed better effect after usage of Dexamethasone dosage (H&E. Magnification at 10X; figure: 9C). The standard control group treated with dexamethasone indicated recovery of lung tissues as a result of being damaged because of disease progression. Hence, Cigarette smoke-induced lung injury rats recovered some of the lung tissues, and some tissues showed distortion of the normal structure.

MgO NPs supplementation with high dose displayed significant improvement in the Cigarette smoke-induced lung injury rats and some of the tissues showed retrieval of their flawless morphological aspects at magnification of 10X. MgO-300 showed significant results after treatment of MgO nanoparticle dosage, almost completely normalized the alveolar structure as compared to low dose treatment group (Figure: 9E). The low dose group treated with MgO-150mg/kg showed partial morphological changes in lung tissues (Figure: 9D).

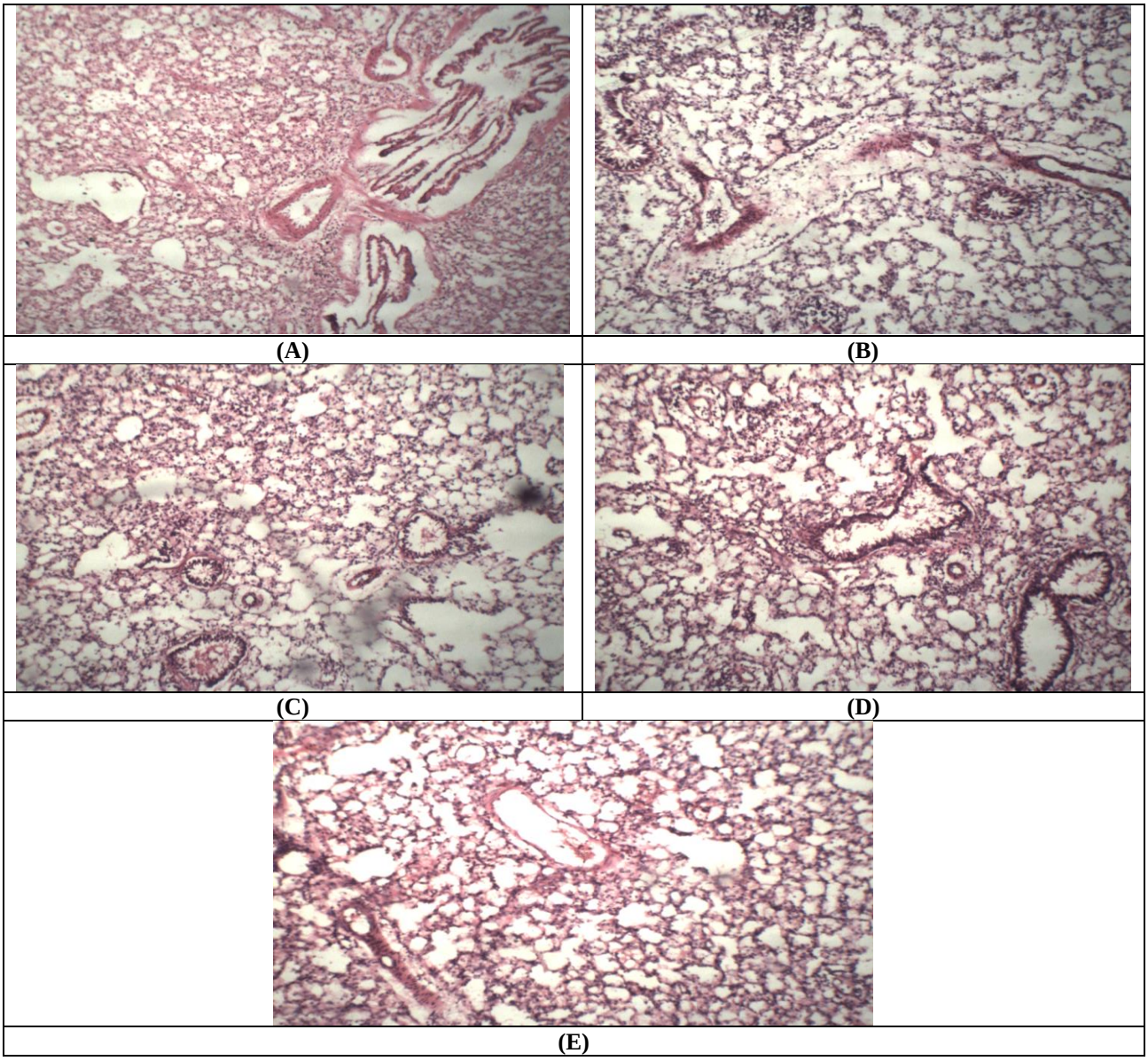



**Figure 9: Histopathology of lung tissue samples**

Negative control group (9A), positive control group (9B), Standard control group (9C), low dose treatment group (9D) and High dose treatment group (9E), at MgO-150mg/kg and MgO-300mg/kg, in Cigarette Smoke induced an acute lung injury-rat model. *MgO= magnesium oxide.

Nanoparticles that are under intense investigation for their potential use in Nano medicine include silver, gold, zinc, copper, and magnesium nanoparticle. MgONPs have been reported to be beneficial for an acute lung injury along with decreasing oxidative stress, and favorable effects. Body weights increases by dosage of MgO supplementation and increase water intake. MgO is also used for the relief of heartburn, sore stomach, and acid indigestion, as an antacid, detoxifying agent, and for bone regeneration (Bertinetti *et al*., 2009).

An MgO-based magnetic tunnel junction sensor together with magnetic NPs is used as biosensors for liver cancer immunoassay (Martinez-Boubeta *et al*., 2010). Different methodologies have been adopted to synthesize nanoparticles, e.g., chemical precipitation, plasma synthesis, aerosol synthesis, sol-gel technique, emulsification, condensation, systemic oligomerization, hydrothermal techniques, etc. (Iravani *et al*., 2014). Zetasizer easy to do and effective and efficient. Acute lung injury in humans is characterized histopathological by neutrophilic alveolitis, injury of the alveolar epithelium and endothelium, increase alveolar surface area and destruction of alveoli. Numerous Nanoparticles in traditional medicine have proven useful for treating various diseases and some of them exhibit significant anti-oxidative potential. The concept of nanoparticle supplementation extensively documented in ancient literature.

The chemical precipitation is a suitable method to be scaled up for large amount of MgO nanoparticles. The size range obtained by chemical precipitation was observed to be 15-35 nanometers and was found to be consistent with the earlier reports by (Balamurugan *et al*., 2015 and Wahab *et al*., 2007)). Similarly, the UV-VIS and zeta sizer analyses were also consistent with earlier studies (Souri *et al*., 2018; Somanathan *et al*., 2016). Another aspect that can be explored is to observe what size and morphology can be created by modulating the chemical reactants and quantitative dynamics there. Normally in our body, there is a balance between the production of free radicals and free radical scavenging, damage repair by free radicals. But with exposure to these MgO nanoparticles to rats, it created an imbalance by depleting or inhibiting the antioxidant system. This may be due to the potential for ROS production (Kiranmai & Reddy, 2013). MgO supplementation of oxidative stress showed favorable result and non-toxic. The human body is continuously bombarded by various harmful chemicals, irritants toxins and infectious entities etc. These inadvertently give rise to a highly unstable class of tractive molecules known as the reactive oxygen species (ROS); these can transfer the vacant and unpaired electrons to various molecular, cellular structures of the cell and resultantly induce damaging oxidation reactions in different cellular compartments.

The assessment of toxicity revealed that, with regards to MgO nanoparticles the groups with dosages of standard, low and high, body weight exhibited lethargy, irritability, decreased food intake, and lack of normal body movements. These observations are in congruence with the earlier studies on MgO nanoparticle toxicity in rat models (Mangalampalli, *et al*., 2017). MgO nanoparticle supplementation in an acute lung injury rat model had a quantifiable and alleviating effect in an acute the lung injury. The major indices including mean body weight, lung injury assessment, liver function assessment; i.e., ALT, AST, kidney function assessment, all exhibited a normalization as contrasted with the positive control group ($P<0.05$). Other parameters also showed a dose-dependent effect on the lung injury status with regards to the MgO nanoparticle supplementation ($P<0.05$). The overall effect of the MgO-300 nanoparticle dosage administration was greater than the other treatment groups ($P<0.05$).

These analyses were quite easy to perform an efficient. Thirty male rats (Wister) were assigned as a positive control group (PCG), negative control group (NCG), standard control group (SCG), low dose (MgO-150) and high dose (MgO-300) treatment group. Each group had six rats and was subjected to standard maintenance conditions. A net gain in body weight was noted in the Standard Control, Low Dose, High Dose Treatment groups ($P<0.05$). The results indicated that body weight increased in groups LD and HD in contrast to the PC group. A decrease in the body weight in the PC group could be due to alveolar destruction and lung pathology. An increase in body weight in treatment groups was a finding, that showed within 21 days in lung injury rats treated with MgO NPs. MgO were chosen because their bulk supplemental form has already been explored for the side effects. BALF indicated that HDTG and LDTG significantly decreased alveolar destruction and derangement of tissues. Positive control group decreased the level of hemoglobin and red blood cells. High dose MgO nanoparticle explored that level of hemoglobin significantly increased ($P<0.05$).

The histopathological examination of lung tissue between all groups were investigated. Negative control group revealed that normal architecture of the lung tissues. Positive control displayed morphological changes in lung tissues. Cigarette smoke-induced lung injury rats treated with MgO NPs recovered remarkable and normalized the lung tissues. It would be fruitful to know which active compounds of the nanoparticle supplementation is responsible for this. Formation of nanoparticles was provided into a dry powder aerosol of micron-sized particles. Stability, ease of handling and

simple inhaler delivery. Inhalable nanoparticulate therapy has great potential (Mansha *et al*., 2023). For the treatment of diseases that require direct pulmonary perfusion. As tuberculosis, with low dose and dose of drug frequency, less systemic side effects and Better patient consent (Sung *et al.*, 2007). Due to the very small size of NPs, these are often prone to drifting into the air and dispersing as environmental pollutants, making the lung more vulnerable to potential exposure as a site of NP interactions. Studies on the transport of NPs in the lung have shown that NPs have the ability to diffuse into the deepest regions of the lung, to important mechanisms such as PS. Given the complexity and important function of PS in regulating healthy lung function, there is a great need to better understand the potential interactions between PS and different types of NPs. Work that has been done in this area so far suggests that NP characteristics such as size, surface charge, and hydrophobicity are critical factors (Arick *et al*., 2015).

## CONCLUSION

The investigation of the acute pulmonary toxicity of MgO NPs in rats after intratracheal administration was done. Different diets do not exhibit exposure to the above-mentioned particles. No mortality was produced in exposed mice. Acute respiratory distress syndrome (ARDS), or acute lung injury (ALI), is a prevalent severe clinical illness in critical care units that may result in a life-threatening type of respiratory failure and a high fatality rate.

## REFERENCES


- Albaiceta, G. M., Brochard, L., Dos Santos, C. C., Fernández, R., Georgopoulos, D., Girard, T., & Blanch, L. (2021). The central nervous system during lung injury and mechanical ventilation: a narrative review. *British Journal of Anaesthesia*, *127*(4), 648-659.
- Altemeier, W. A., Hung, C. F., & Matute-Bello, G. (2017). Mouse models of acute lung injury. *Acute Lung Injury and Repair: Scientific Fundamentals and Methods*, 5-23.
- Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). Moringa oleifera: a food plant with multiple medicinal uses. Phytotherapy Research: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *21*(1), 17-25.
- Arick, D. Q., Choi, Y. H., Kim, H. C., & Won, Y. Y. (2015). Effects of nanoparticles on the mechanical functioning of the lung. *Advances in Colloid and Interface Science*, *225*, 218-228.
- Balamurugan, M., Venkatesan, G., Ramachandran, S., & Saravanan, S. (2015). Synthesis and Characterization of Manganese Oxide Nanoparticles. *Synthesis and Fabrication of Nanomaterials*, 311-314.
- Bertinetti, L., Drouet, C., Combes, C., Rey, C., Tampieri, A., Coluccia, S., & Martra, G. (2009). Surface characteristics of nanocrystalline apatites: effect of Mg surface enrichment on morphology, surface hydration species, and cationic environments. *Langmuir*, *25*(10), 5647-5654.
- Bindhu, M. R., Umadevi, M., Micheal, M. K., Arasu, M. V., & Al-Dhabi, N. A. (2016). Structural, morphological and optical properties of MgO nanoparticles for antibacterial applications. *Materials Letters*, *166*, 19-22.
- Bonner, J. A., Harari, P. M., Giralt, J., Cohen, R. B., Jones, C. U., Sur, R. K., Zhu, J. (2010). Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. *The lancet oncology*, *11*(1), 21-28.
- Cai, L., Chen, J., Liu, Z., Wang, H., Yang, H., & Ding, W. (2018). Magnesium oxide nanoparticles: effective agricultural antibacterial agent against Ralstonia solanacearum. *Frontiers in microbiology*, *9*, 790.
- Dianat, M., Radan, M., Badavi, M., Mard, S. A., Bayati, V., & Ahmadizadeh, M. (2018). Crocin attenuates cigarette smoke-induced lung injury and cardiac dysfunction by anti-oxidative effects: the role of Nrf2 antioxidant system in preventing oxidative stress. *Respiratory research*, *19*(1), 1-20.
- Donaldson, K., Aitken, R., Tran, L., Stone, V., Duffin, R., Forrest, G., & Alexander, A. (2006). Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. *Toxicological sciences*, *92*(1), 5-22.
- Dreher, M., Kersten, A., Bickenbach, J., Balfanz, P., Hartmann, B., Cornelissen, C., & Marx, N. (2020). The characteristics of 50 hospitalized COVID-19 patients with and without ARDS. *Deutsches Ärzteblatt International*, *117*(16), 271.
- Gelli, K., Porika, M., & Anreddy, R. N. R. (2015). Assessment of pulmonary toxicity of MgO nanoparticles in rats. *Environmental toxicology*, *30*(3), 308-314.
- Iravani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of silver nanoparticles: chemical, physical and biological methods. *Research in pharmaceutical sciences*, *9*(6), 385.
- Kim, H. W., Ahn, E. K., Jee, B. K., Yoon, H. K., Lee, K. H., & Lim, Y. (2009). Nanoparticulate-induced toxicity and related mechanism in vitro and in vivo. *Journal of Nanoparticle Research*, *11*(1), 55-65.
- Kiranmai, G., & Reddy, A. R. N. (2013). Antioxidant status in MgO nanoparticle-exposed rats. *Toxicology and industrial health*, *29*(10), 897-903.
- Mangalampalli, B., Dumala, N., & Grover, P. (2017). Acute oral toxicity study of magnesium oxide nanoparticles and microparticles in female albino Wistar rats. *Regulatory Toxicology and Pharmacology*, *90*, 170-184.
- Muhammad Tamoor Mansha, Ayesha Maqbool, Nabeela Nadir, Ali Imran Mallhi, Sehar Arshad, Maira Munir, FahadAli Nadeem, Mubashir Ahmad,

Aftab Hussain (2023). Functional Assessment of MGO Nanoparticle Supplementation in an AcuteLiver Injury Rat Model. Haya Saudi J Life Sci, 8(11): 233-241.

- Martinez-Boubeta, C., Balcells, L., Cristòfol, R., Sanfeliu, C., Rodríguez, E., Weissleder, R., Sandiumenge, F. (2010). Self-assembled multifunctional Fe/MgO nanospheres for magnetic resonance imaging and hyperthermia. *Nanomedicine: Nanotechnology, Biology and Medicine*, *6*(2), 362-370.
- Matthay, M. A., & Zimmerman, G. A. (2005). Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. *American journal of respiratory cell and molecular biology*, *33*(4), 319-327.
- Rafiei-Sarmazdeh, Z., Zahedi-Dizaji, S. M., & Kang, A. K. (2019). *Two-dimensional nanomaterials*. London, UK: IntechOpen.
- Sasannejad, C., Ely, E. W., & Lahiri, S. (2019). Long-term cognitive impairment after acute respiratory distress syndrome: a review of clinical impact and pathophysiological mechanisms. *Critical care*, *23*(1), 1-12.
- Somanathan, T., Krishna, V. M., Saravanan, V., Kumar, R., & Kumar, R. (2016). MgO nanoparticles for effective uptake and release of doxorubicin drug: pH sensitive controlled drug release. *Journal of Nanoscience and Nanotechnology*, *16*(9), 9421-9431.
- Souri, M., Hoseinpour, V., Shakeri, A., & Ghaemi, N. (2018). Optimisation of green synthesis of MnO nanoparticles via utilising response surface methodology. *IET nanobiotechnology*, *12*(6), 822-827.
- Stine, J. G., & Lewis, J. H. (2016). Current and future directions in the treatment and prevention of drug-induced liver injury: a systematic review. *Expert review of gastroenterology & hepatology*, *10*(4), 517-536.
- Sung, J. C., Pulliam, B. L., & Edwards, D. A. (2007). Nanoparticles for drug delivery to the lungs. *Trends in biotechnology*, *25*(12), 563-570.
- Wahab, R., Ansari, S. G., Dar, M. A., Kim, Y. S., & Shin, H. S. (2007, October). Synthesis of magnesium oxide nanoparticles by sol-gel process. In *Materials Science Forum* (Vol. 558, pp. 983-986). Trans Tech Publications Ltd.
- Zemans, R. L., Colgan, S. P., & Downey, G. P. (2019). Transepithelial migration of neutrophils: mechanisms and implications for acute lung injury. *American journal of respiratory cell and molecular biology*, *40*(5), 519-535.