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Research Article

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Therapeutic Potential of Sub- Acute MgO Nanoparticle Exposure Against Lungs Injury Associated Systemic Complications in Rat Model

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Abstract

Acute lung injury is characterized by acute inflammation and disruption of the lung endothelial and epithelial barriers. Acute Respiratory Distress Syndrome or ARDS clinically characterized 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. This study was designed to investigate the ameliorative effect of MgO Nanoparticle, on body weight, BALF, antioxidant activity, liver, lipid profile, complete blood count and lungs in cage cigarette smoke acute lung injury rat model. Thirty albino healthy male rats were randomly divided into five experimental groups, 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). Results revealed that MgO nanoparticles exhibited dose dependent increase in body weight, reduced inflammatory cell infiltration in alveoli, normalized antioxidant capacity, attenuate hepatic damage, reduced serum total cholesterol, triglycerides, HDL-c LDL-c, increased peripheral blood WBCs, RBCs, Hb and platelets, attenuate the lungs histopathological alterations, progressive enlarged alveolar space, bronchial constriction, dilation and thinning of pulmonary vessel. Intra-alveolar multinucleated macrophages accumulation, apoptosis and deterioration of alveolar walls in" cage cigarette smoke acute lung injury rat model.

Conclusively this formulation of selected nanoparticles at high dose exhibited dose dependent potential ameliorative effect on acute lung injury and can be valuable therapeutic intervention.

Keywords: MgO nanoparticle; Body weight; Cigarette smoke; Histopathological; Inflammation

INTRODUCTION

Acute lung injury (AcLI) is characterized by acute lungs inflammation, disruption of the endothelial and epithelial barriers. The alveolar–capillary membrane is comprised of the microvascular endothelium, interstitial, and alveolar epithelium. Cellular characteristics of AcLI include loss of alveolar–capillary membrane integrity, excessive trans-epithelial neutrophil migration, and release of pro-inflammatory, cytotoxic mediators [1].

Lung injury and the acute respiratory distress syndrome (ARDS) describe clinical syndromes of acute respiratory failure with substantial morbidity and mortality. Even patients surviving with acute lung injury, there is evidence that their long-term quality of life is adversely affected [2]. The American/European Consensus Conference defined patients 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 (AcLI) or less than 200 (ARDS) [3]. Clinical acute lung injury is associated with specific risk factors broadly divided into intra-pulmonary conditions including pneumonia, aspiration, and blunt trauma; and extra-pulmonary risk factors, including extra-pulmonary sepsis, trauma, and pancreatitis. Several clinical disorders can initiate ARDS, including pneumonia, sepsis, gastric aspiration, and trauma [4]. 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 [5].

There are many potential paths in bio-nanotechnology being targeted to treat lung injuries. Numerous nanoparticles, such as zinc oxide, casein, and different polymers, have been studied for their potential to attenuate lung injury. The design and testing of many nanoparticles for oral delivery of treatment for lung injury is now hot area of research [6]. Clinically, ARDS is manifested by bilateral or diffuse radiographic infiltrates, hypoxemia, decreased lung compliance, and increased ventilatory dead space [7]. Similarly, NPs exhibit unusual adsorptive properties, fast diffusivity and unstable in critical conditions [8].

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 surface charege, making them a promising tool for biomedical applications [9]. Recent studies, aim to understand the toxicity mechanism of MgO NPs, showed physical injury of cell membranes, decreased motility and biofilm formation ability of R. solanacearum, mediated by direct attachment of MgO NPs to bacterial cells surface, observed under scanning electron microscopy (SEM) and transmission electron microscopy [10].

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MgO exposure showed the dose-dependent infiltration of interstitial lymphocytes, lymphoid aggregation, dilated congested vessels, peribronchiolar lymphocytic infiltration, granulomatous reactions, and alveolar macrophages at 1day post-exposure and were worsened at 1-week period, reduced cured at 1-month post-instillation period, supporting the lung damage, which was comparable to Quartz NPs. The above results, suggested that intratracheal instillation of these MgO NPs produced a significant increase in pulmonary enzymatic levels, which was supported by histopathological examinations, indicated 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 [11].

The pulmonary toxicity of MgO NPs in rats was mediated by the generation of ROS. Exposure of rats to MgO NPs induce depletion or inhibition of the antioxidants and increased the formation of ROS through mitochondria dysfunction. Many evidences showed that NPs increase ROS production and cell death in different types of cell culture. Previously reported that elevated levels of MDA, depletion of glutathione, Catalase, SOD, and TAS activity are strongly correlated to ROS generation and lipid peroxidation, indicated the induction of marked oxidative stress in rats [12].

Neutrophils adhering to the injured capillary endothelium and margination through the interstitial into the air spaces, filled with protein-rich edema fluid has been reported. In the air space, an alveolar macrophage, secreting cytokines, interleukin (IL)-1, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α, 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) [13].

MgO has been used for heartburn relief, sore stomach, and acid indigestion, as an antacid, detoxifying agent, and bone regeneration [14]. The previous studies have not been reported the in vivo dose dependent effect of MgO NPs nanoparticles. The purpose of this study was to explore the dose dependent ameliorative effect of MgO NPs in cage cigarette smoke model of acute lung injury rat model.

MATERIAL AND METHODS

Ethical approval

The study was conducted in Koc University graduate school of health sciences. All methods were performed in accordance with relevant guidelines and regulations of "local ethics committee for animal experiments of Koc university." The study was approved by the committee with Approval No. (2023-5). The rats were kept in the Koc University, Animal Research Facility (KUARF) of Centre for Translational Medicine (KUTTAM). All of animal procedures were carried out under the standard rules established by the Governing Board of the National Research Council, whose members are drawn from the Institute of Medicine. The members of the Committee responsible for the study were chosen for their special competences and with regard for appropriate balance.

Chemicals

Magnesium oxide nanoparticle (MgO NPs) were synthesized in our laboratory at KUTTUM, Cigarettes were purchased from Gold Flake Cigarettes, Company, Pakistan Tobacco Co. Ltd. (nicotine: 6.17 mg), smoke generating chamber was available in laboratory, Trizol ,70% ethanol, NH3, Mg (NO3)2 and nitric acid were purchased form Sigma-Aldrich Co. (USA). Ketamine HCl (10%) from Alfasan Co. (Netherlands). DexaCare (Dexamethasone) was purchased from Adva Care Pharma Pvt. Ltd.

Experimental animals

The thirty (30) albino male rats of 150-300 grams were obtained from Koc University, Animal Research Facility (KUARF). Albino rats were divided into five groups allowed to acclimatize and were kept in well-aerated cages with free access to a chow maintenance diet (CMD) and water at a temperature of 25±2 ºC.

Animal Diet

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.

Study design

Establishment of CS-Induced Lung Injury Model

Negative control group (NCG) was provided with fresh air and four groups were affected by Cigarette Smoke. In the CS- induced lung injuries model group, the rats were completely exposed to smoke during trial on daily basis, four cigarettes per day.

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 [15].

Instillation of CS-induced lung injury and smoke exposed rats

Albino rat's groups were intratracheally instilled with, PBS, Low dose of MgO 150 mg/kg in distilled water, high dose of MgO 300mg/kg in distilled water. The first group negative control (NC), was instilled with PBS, second positive control (PC; CS-induced lung injury model) was instilled with PBS, third standard control (SC) was instilled with Dexamethasone 1mg/ml with PBS. The fourth and fifth group were instilled MgO nanoparticle dosages of (LDG-MgO 150mg/kg) and (HDG-MgO 300mg/kg) in distilled water respectively for 21days. The PBS and MgO particle administered rats were subjected to BALF (bronchoalveolar lavage fluid) analysis, antioxidant, liver function, complete blood count and histopathological analysis at 21th day of exposure as listed in Table 1.

Table 1: Experimental design; *CMD=chow maintenance diet; n=number of rats; CG = Cigarettes.

Serial No.	Groups	ID	Daily Dosage and instillation (mg/kg)
1	Negative Control group	NCG	CMD+ Water. No smoke exposure (N=6) **Instillation: PBS**
2	Positive Control group	PCG	CMD+ Cigarette smoke exposure: CG 4/day (N=6) **Instillation: PBS**
3	Standard Control group	SCG	CMD+ Cigarette smoke exposure: CG 4/day (N=6) **Instillation: Dexamethasone 1mg/ml +PBS**
4	Low dose treatment group	LDTG	CMD+ Cigarette smoke exposure: CG 4/day (N=6) **Instillation: DW+ MgO 150mg/kg/day**
5	High dose treatment group	HDTG	CMD+Cigarette smoke exposure: CG 4/day (N=6) **Instillation: DW + MgO 300mg/kg/day**

Sample collection

At 21th day of treatment, samples were collected. At the end of experimental trials, the animals were euthanized, organ and tissue samples were collected (i.e. Lungs) and preserved in neutral buffered formalin for histopathology. Blood samples were collected 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 injury

The procedure of Bronchoalveolar Lavage Fluid (BALF)

An established method for examining the cellular components of the airways and air gaps is bronchoalveolar lavage (BAL). A catheter was inserted into the rat's trachea and a saline solution was infused into the bronchioles before the infused fluid is slowly pulled to isolate BAL fluid [15].

Rats were initially cut into the neck region for exposing the trachea and lungs by slicing 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 filled with the normal saline. The fluid 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. 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 Eppendorf tubes and rapidly frozen at -40 °C for TAC, TOS, Inflammatory cells analysis and MDA assays.

Inflammatory cell analysis in BALF

Inflammatory cells were quantified in BALF by previously established method [16]. Immediately after euthanization 1ml of 0.9% BALF was collected for total cell enumeration. The total quantity of cells in BAL was counted and 2×10^{2} cells were centrifuged onto glass slides at 800 rpm. Hema3 kit (Biochemical Sciences, Inc., Swedesboro, NJ) was for staining Cytospins and 600 cells used for differential cell counts.

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) was used 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 [17].

Malondialdehyde (MDA; nmol/ml)

Malondialdehyde (MDA) serves as the primary indicator of lipid peroxidation as it is 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. 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 µl) 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 °C for 1 hour for incubation. After 1 hour of incubation, all the Eppendorf tubes were chilled at room temperature, after that 625 µl of Butanol (from prepared Butanol & Pyridine solution) was added. All 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 absorbance of the supernatant was measured at 532 nm.

Liver function analysis

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

Complete blood count analysis

On same day of sample collection, sample were referred to hematology lab for complete blood count analysis. CBCs analysis was carried out on SemiAutomatic Haematology Analyzer Mindray CBC Machine.

Histopathological examination

The albino rat's lungs were exposed to PBS, Smoke, (PC; CS-induced lung injury model) with PBS, Dexamethasone with PBS, MgO nanoparticle dosages of (LDG-MgO 150mg/kg) and (HDG-MgO 300mg/kg) lungs were prepared for microscopic analysis. Lungs tissue were dissected from right lower regions and embedded in paraffin.5µm thick sagittal sections were expurgated with microtome, mounted and stained (hematoxylin-eosin). Prepared slides were subsequently observed under light microscope.

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 Graph Pad Prism 8.0 and Co-Stat computer software.

RESULTS

The data was analyzed at two phases. In first phase, characterization, Zetasizer and Ultraviolet Visible Spectroscopic Analysis was performed and in second phase, the data was analyzed statistically in tabulation form represented by description.

MgO nanoparticle synthesis and characterization

MgO nanoparticles were synthesized by methods described [18]. The synthesis process completed in two phases. The first phase was Mg(OH)2 precipitation followed its calcination at different temperature range until magnesium oxide was formed. Mg(OH)2 was obtained by adding aqueous NH3 to Mg (NO3)2 solution mixed and precipitated, by

continuous stirring with magnetic stirrer. The Mg (NO3)2 (0.217M) was obtained by dissolving three times more MgO then nitric acid in solution. The resulting precipitate was washed with dH2O and lamp dried in filter paper at 40–50°C for characterization.

Zetasizer Analysis of Magnesium Oxide (MgO) Nanoparticles

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

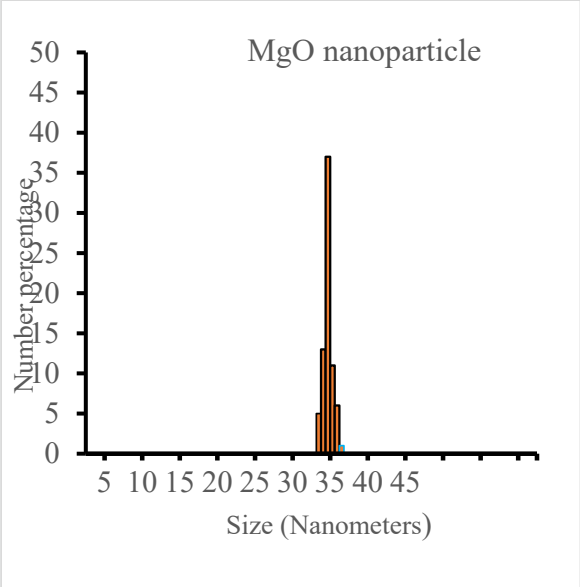

Figure 1 The average size of MgO nanoparticles

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 representing magnesium oxide nanoparticles (Figure 2). The finding was in agreement with another study [19].

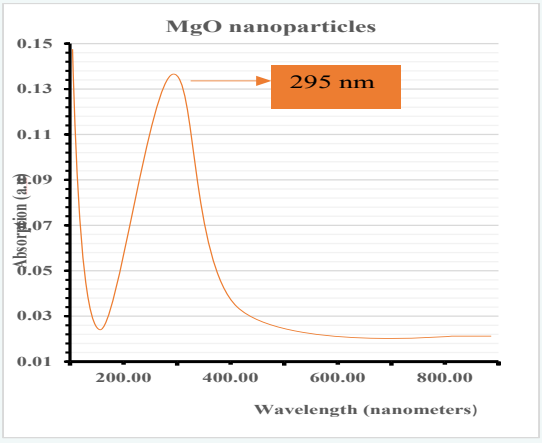

Figure 2 Magnesium oxide nanoparticles

Mean Body Weight Analysis

The body weight of each group was evaluated at day 7, 14 and 21 using one-way analysis of variance and found significant variations between negative control and high dose treated groups on 21th day (Figure 3; $P \leq 0.05$). A significant increase in mean body weight of treated groups, i.e., LDG, HDG after the 2nd week of the trial was observed. According these observations, the positive control group revealed a statistically significant decreased in mean body weight ($P<0.05$) compared to NCG and HDTG. A significant increase in mean body weight was recorded in the (LDG-MgO 150) and (HDG-MgO 300) and groups at day 21 ($P<0.05$).

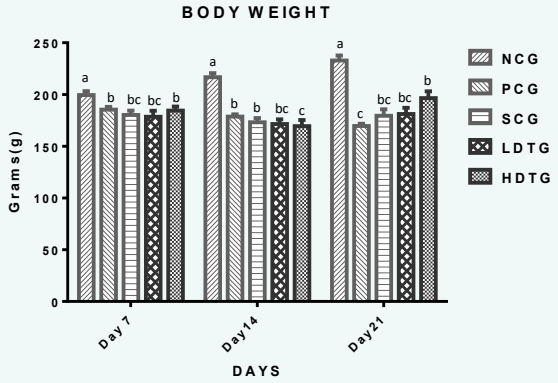

Figure 3 Effect of MgO nanoparticle on Body weights of cigarette smoke induced acute lung injury-rat model. The mean body weight (grams ±SE) of NCG, PCG), Standard Control Group SCG, LDTG-MgO 150 and HDTG-MgO 300 at day 7, day14, day 21 are depicted in figure. The mean body weight of (HDTG-MgO 300) CS- lung injury rats model was significantly increased compared to other experimental group at 21 th day. Means values were significant at ($P \leq 0.05$).

Bronchoalveolar Lavage Fluid Analysis (BALF)

The Bronchoalveolar Lavage Fluid Analysis (BALF) evaluated among all experimental groups. The PCG showed statistically significant increased macrophage, lymphocytes, neutrophil, eosinophil and Th cells. However, the eosinophils and Th cells counts were not higher than other inflammatory cells. One can have noticed the inflammation mediated alveolar destruction and surface area in photomicrograph of PCG was significantly higher ($P<0.05$) than LDTG and HDTG. The marked reduced cell counts in High Dose Treatment Group (HDTG) associated with reduced inflammatory induced alveolar destruction and increased surface area at day21 (Figure 4).

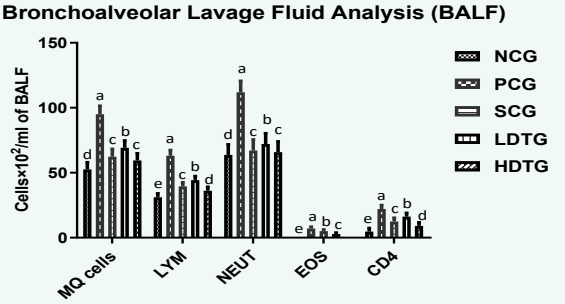

Figure 4 Effect of MgO nanoparticle on serum concentration of Bronchoalveolar Lavage Fluid (cells×102/ml). Different cells count of Bronchoalveolar Lavage Fluid (BALF) in Negative Control Group (NCG), Positive Control Group (PCG), Standard Control Group (SCG), Low Dose Treatment Group (LDTG-MgO 150), High Dose Treatment Group (HDTG-MgO 300). The inflammatory cell counts in (LDTG-MgO 150) and (HDTG-MgO 300) BALF were significantly reduced compared to positive control.

Total antioxidant capacity, total oxidant status, malondialdehyde assay

Total antioxidant capacity analysis revealed significant difference among PCG, LDTG, and HDTG (P<0.05). Mean BALF, TAC level of HDTG-MgO-300 group was significantly decreased compared to PCG, (LDTG-MgO 150) and (HDTG-MgO 300) (Figure 5A; P<0.05). One-way ANOVA was used to determine the BALF, TOS level in each group. Decreased total oxidant status (TOS) in High Dose Treatment Group was observed compared to Positive Control Group (PCG) and Low Dose Treatment Group (LDTG) (Figure 5B; P<0.05). The analysis of serum MDA levels among different groups revealed significant decreased MDA concentration in SCG, LTDG and HDTG. (Figure 5C; P≤0.05).

High dose MgO nanoparticles ameliorated the liver injury in SC- lung injury rat-model demonstrated by decreased Aspartate Aminotransferase (AST; U/L) and Alanine transaminase (ALT; U/L)

In order to investigate the effect of low and high dose MgO nanoparticles on liver functions of CS- acute lung injury rat-model the serum aspartate aminotransferase (AST) concentration was performed by Bio-active kit AST KITLOTBD80932 (U/L). The mean concentration of aspartate aminotransferase was significantly increased in PCG as compared to LDG and HDG (Figure 6B; p<0.05). These finding suggested that acute lung injury also effects liver function and ameliorated by high dose MgO nanoparticles.

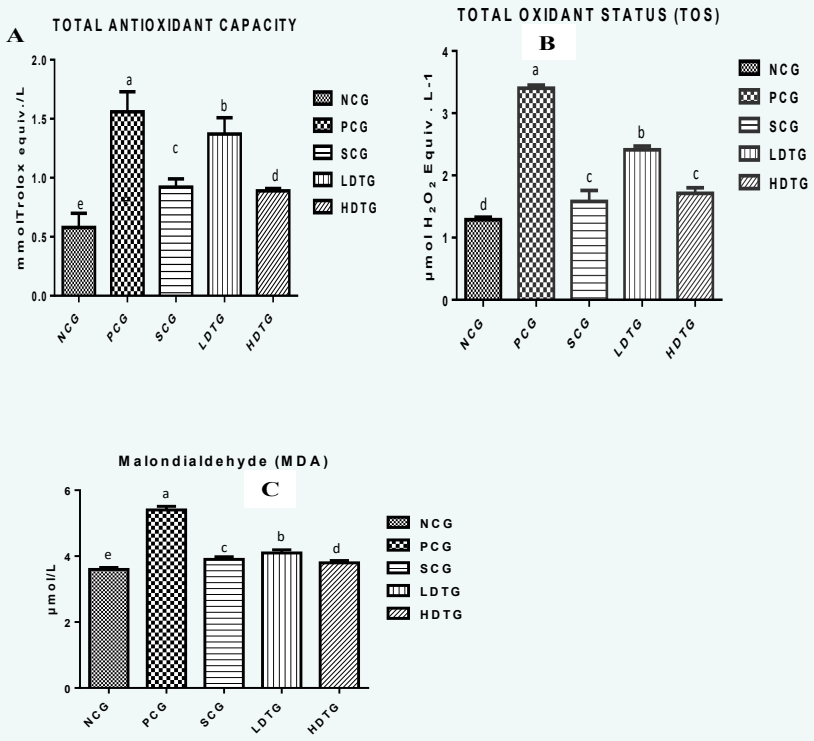

Figure 5: Effect of MgO nanoparticle on serum concentration of TAC (5A; mmol Trolox equivalent/L), TOS (5B; µmol H_2O_2 Equiv.L^{-1}) and MDA (5C; nmol/L). The results are demonstrated as means ± SE. Mean values significantly differed are represented as (P≤0.05). (A, B, C).

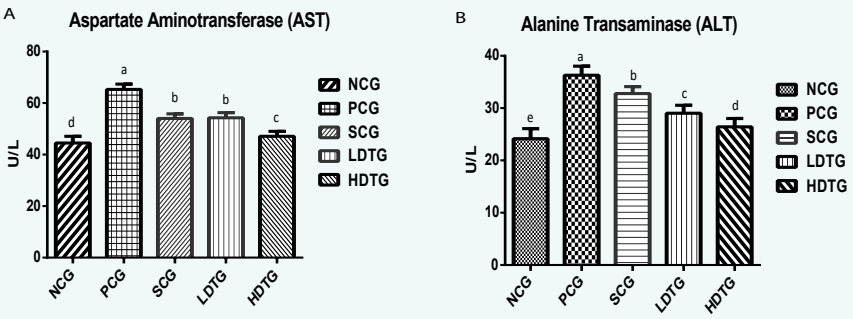

Figure 6: Effect of MgO Nanoparticles on serum concentration of AST (µ/l; Figure 6A) and ALT (u/l; Figure 6B). Results are presented as means ± SE. The significant decreased serum AST and ALT was observed in SCG, LDTG-MgO 150 and HDTG-MgO 300. HDTG-MgO 300mg/kg compared to PCG (CS-lung injury) showed more significant lung injury associated hepatic damage recovery compared to LDTG-MgO 150 mg/kg in CS- lung injury rat-model (P≤0.05) **(A, B).**

Lipid profile

Total Cholesterol (mg/dl), Serum Triglyceride (mg/dl), Serum Low Density Lipoprotein (LDL) and Serum High Density Lipoprotein (HDL) alteration in acute lung injury and improvement upon sub-acute exposure of MgO nanopartcles

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 mean level of total cholesterol (TC) was significantly decreased in treatment groups as compared to the PCG (Figure 7A; P≤0.05). Bio-active kit was used (KIT LOT BD601032) to analyze the serum triglyceride level. Mean Serum Triglyceride level analysis demonstrated a statistically significant decreased triglyceride in low and high dose treated groups compared to positive control. (Figure 7B; P<0.05). ANOVA analysis of LDL-Cholesterol levels among different groups demonstrated a significant decrease in treatment groups as compared to the positive control (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).

Hematological Parameters

In this study different blood parameters such as Hemoglobin, Platelets, Red Blood Cell, White Blood Cells were determined.

Hematological parameters Red Blood Cells (RBC=x10^6/ cubic millimeter), Hemoglobin (g/dl), White Blood Cells (WBC=x10^3/ mm^3) (µL) and platelets (µL) alteration in acute lung injury and amelioration by sun-acute exposure to MgO nanoparticles

One-Way analysis of variance was used to determine the blood red blood cells (RBCs) in all 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 was 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). HDG-MgO 300mg/kg showed, increased level of hemoglobin 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).

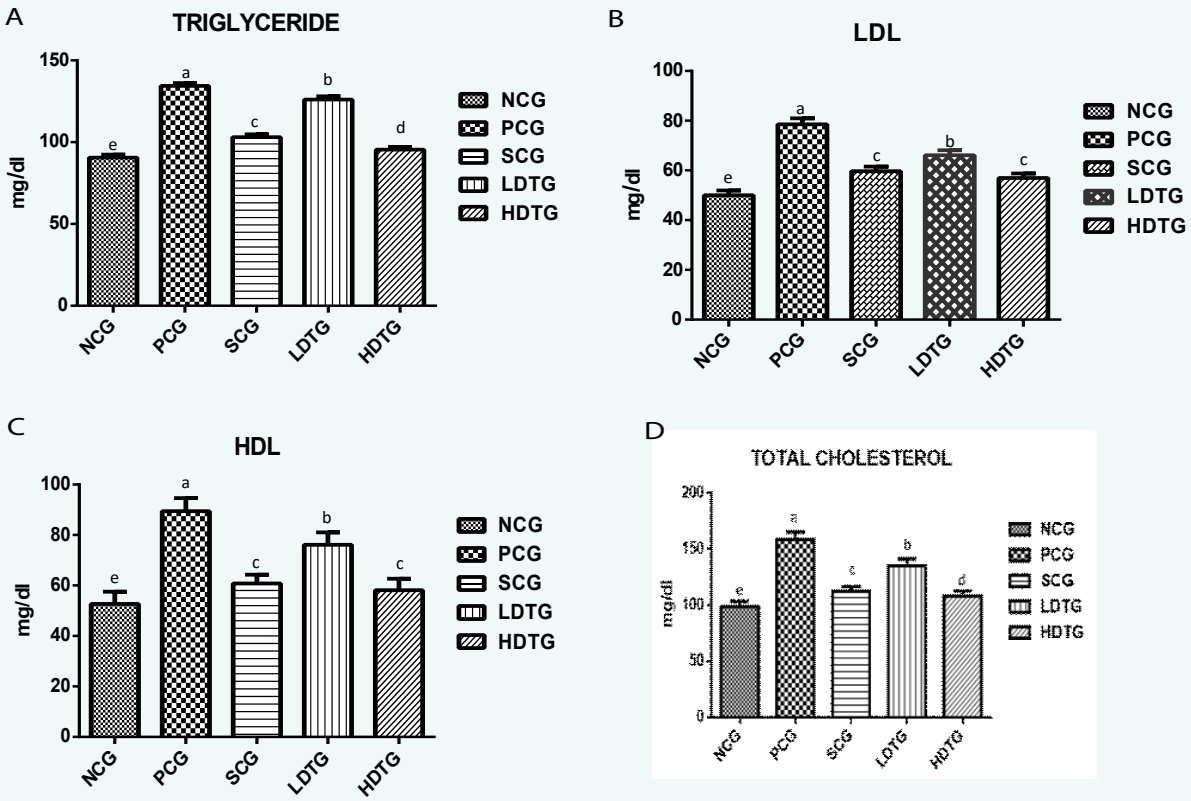

Figure 7: Effect of MgO NPs on serum concentration of TC (7A; mg/dl), TG (7B; mg/dl), LDL (7.C) and HDL (7. D). Results are presented as means ± SE level of significance was (P≤0.05). The serum level of TC, TG, LDL-cholesterol and HDL-cholesterol was significantly reduced in SCG, LDTG-MgO 150 and HDTG-MgO 300 compared to PCG (CS-acute lung injury rat-model) **(A, B, C, D).** The reduction was more marked HDTG-MgO 300 compared LDTG-MgO 150 (P≤0.05).

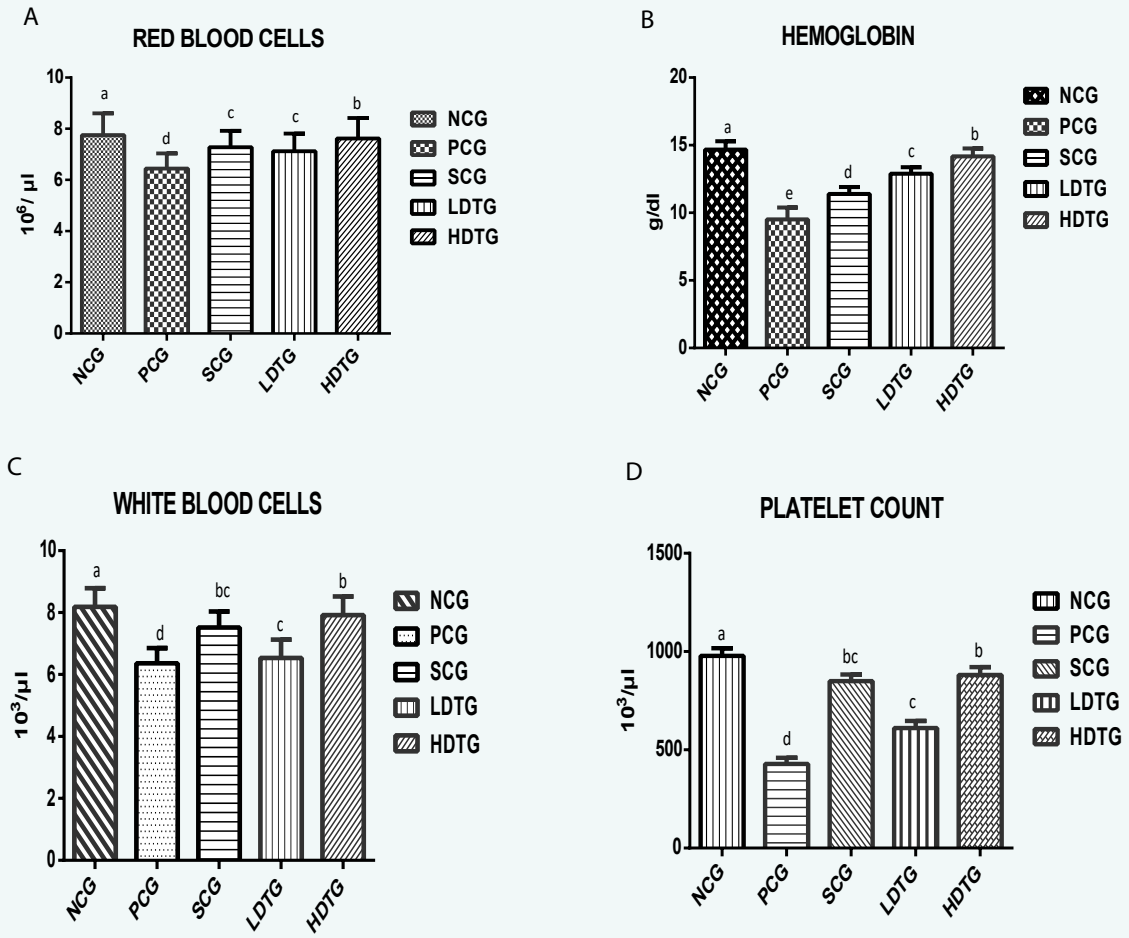

Figure 8: Demonstrated the Effect of MgO nanoparticle Red blood cells (µl; 8A), Hemoglobin (g/dl; 8B), white blood cells (µl; 8C) and platelets count (µl; 8D)

Results are depicted as means ± SE. RBCs count was significantly increased following the lungs exposure to dexamethasone (SCG), LDTG-MgO 150 and HDTG-MgO 300 compared to PCG (CS-acute lung injury rat-model). P value (P≤0.05) was significant. Hemoglobin concentration elevated in dexamethasone (SCG), LDTG-MgO 150 and HDTG-MgO 300 compared to PCG **(A, B).** WBCs and platelets count was also significantly increased in dexamethasone (SCG), LDTG-MgO 150 and HDTG-MgO 300 compared to PCG however count increment was less increased in LDTG-MgO 150 compared to HDTG-MgO 300 **(C, D).**

White blood cells (WBCs) level were compared with different groups by using ANOVA. The serum level of WBCs was significantly decreased in PCG compared to NCG i.e., 6.36±0.49 as compared to 8.19±0.60 (P≤0.05). HDG-MgO 300mg/kg revealed significantly increased serum WBCs level as compared to PCG 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).

Cigarette Smoke induced acute lung histopathological damage and attenuation by dose dependent sub-acute exposure of MgO nanoparticles

The MgO nanoparticle administration in" Cigarette Smoke induced acute lung injury rat model" revealed potential attenuation of lung injury following sub-acute exposure to dexamethasone, LDTG-MgO 150 mg/kg and HDTG-MgO 300 mg/kg. The negative control indicated normal architecture of the lung histology (Figure 9A). The positive control showed a progressive change along with structural derangement in the tissue. Progressive enlarged alveolar space, bronchial constriction, dilation and thinning of pulmonary vessel. Intra-alveolar multinucleated macrophages accumulation within alveolar spaces, apoptosis and deterioration of alveolar wall was examined in microscopic analysis. Hematoxylin and Eosin stained lungs tissue of experimental groups are analyzed and compared (H&E magnification at 10X Figure 9B). Dexamethasone exerts a less potent recovery effect on CS- injured lungs (Figure: 9C). MgO-300 significantly recovered lung tissue injury as normalized alveolar space and marked bronchial lumen dilation (Figure 9D). Sub-acute exposure of MgO NPs at high dose displayed significant improvement marked by almost normal alveolar space, alveolar wall thickness, and bronchial lumen diameter in CS-induced lung injury rats (Figure 9E).

DISCUSSION

The aim of this study was to investigate the ameliorative effect of sub-acute MgO NPs exposure against Cigarette Smoke induced acute

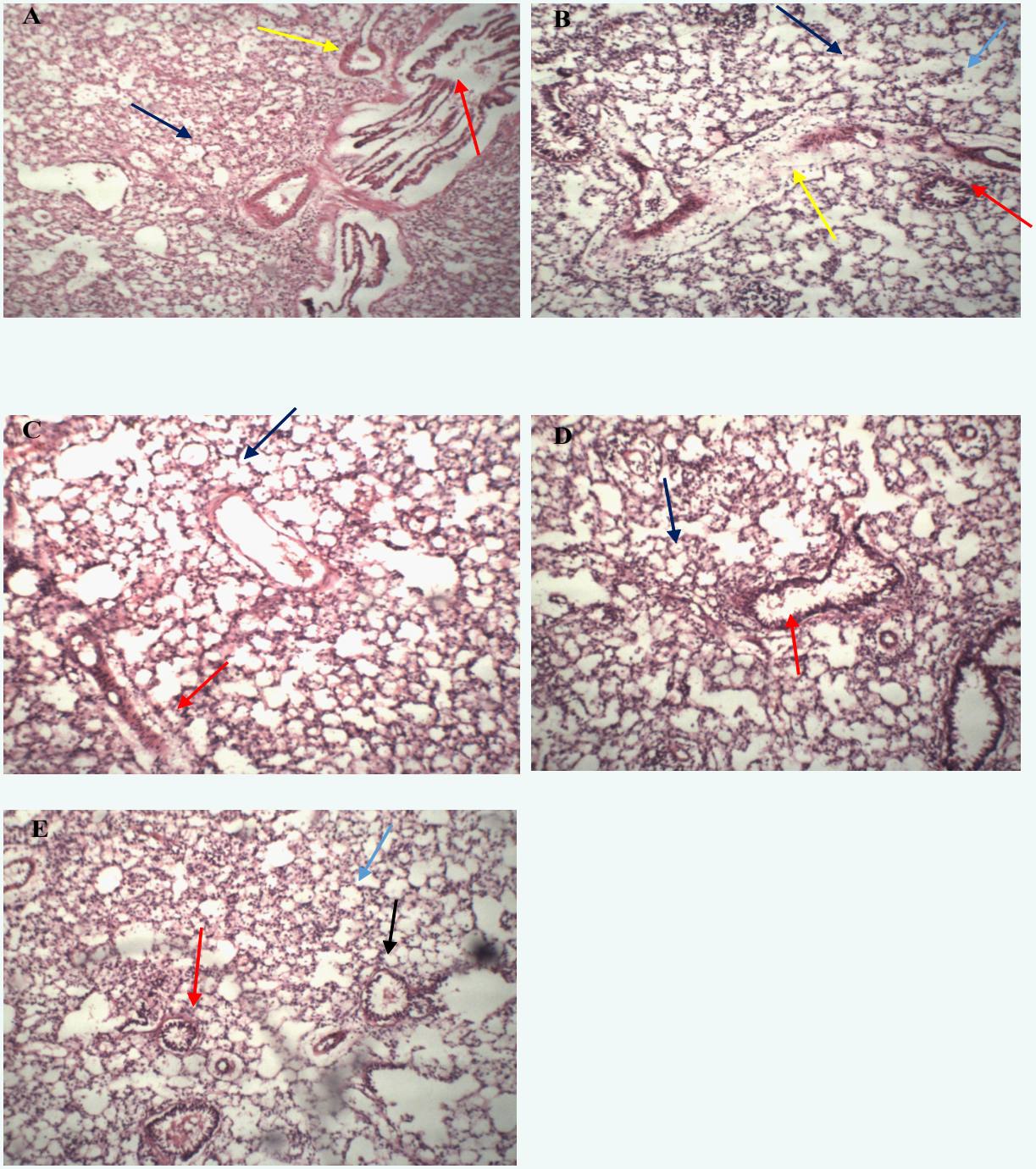

Figure 9: Illustrated the Effect of MgO nanoparticle on histopathology of lung tissue stained with hematoxylin and eosin (H and E)

Histopathology of lung tissue stained with hematoxylin and eosin (H and E). A negative control (NCG) showed normal alveolar spaces (dark blue arrow), normal bronchial lumen (yellow arrow), pulmonary vessel is not evident **(9A).** In positive control group (PCG) rats exposed to cigarette smoke induced lung injury showed progressive increasing alveolar space (dark blue arrow), bronchial constriction (red arrow), dilation and thinning of pulmonary vessel, (yellow arrow). Intra-alveolar multinucleated macrophages accumulation within alveolar spaces, apoptosis and deterioration of alveolar wall was examined **(9B).** Standard control group (SCG) rats treated with Dexamethasone showed marked decreased alveolar space (dark blue arrow), recovered alveolar wall thickness, increased pulmonary vessel wall thickness, in CS- lung injury-rat model (black arrow) **(9C).** Low dose treatment group (LDG- MgO 150) showed significant normalized alveolar space (dark blue arrow) and marked bronchial lumen dilation (red arrow) **(9D).** High dose treatment group (HDG- MgO 300) revealed almost normal alveolar space, alveolar wall thickness (blue arrow) and bronchial lumen (red and black arrow) at 21th day of treatment **(9E).** *MgO magnesium oxide.

lung injury-rat after intratracheal administration. Previous findings have reported that acute respiratory distress syndrome (ARDS), or acute lung injury (ALI), is a prevalent severe clinical illness in critical care units that may results a life-threatening type of respiratory failure and a high mortality rate of up to 30–40%. Nanoparticle have been employed for in vivo diagnosis by concentrating, safeguarding, and enhancing a biomarker from the breakdown that can in turn, yield accurate and sensitive data. Nanoparticles that are under intense investigation for their potential use in Nano medicine include silver, gold, zinc, copper, and magnesium nanoparticle. Bulk forms of MgO have been reported to be beneficial for an acute lung injury along with decreasing oxidative stress, and promising systemic effects. Body weights increases by dosage of MgO supplementation and increase water intake due to bulk form of NPs. MgO is also used for the relief of heartburn, sore stomach, and acid indigestion, as an antacid, detoxifying agent, and for bone regeneration [20].

Our finding revealed that sub-acute exposure to LDT-MgO 150 mg/kg and HDT-MgO 300 mg/kg in CS- induced acute lung injury-rat results ameliorated dysfunctions manifested as increasing body weight, reduced inflammatory cell infiltration in alveoli, normalized antioxidant capacity, attenuate hepatic damage presented by decreased level of aspartate aminotransferase(AST) and alanine transaminase (ALT), reduced serum total cholesterol, triglycerides, HDL-c LDL-c, increased peripheral blood WBCs, RBCs, Hb and platelets, attenuates the lungs histopathological alterations, marked by progressive enlarged alveolar space, bronchial constriction, dilation and thinning of pulmonary vessel. Intra-alveolar multinucleated macrophages, lymphocytes, neutrophil, eosinophil and Th cells accumulation, apoptosis and deterioration of alveolar walls.

An MgO-based magnetic tunnel junction sensor together with magnetic NPs is used as biosensors for liver cancer immunoassay [21]. 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 [22].

Zetasizer easy to perform, 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 [23,24]. Similarly, the UV-VIS and zeta sizer analyses were also consistent with earlier studies [25,19]. Another aspect that can be explored is to observe what size and morphology can be created by modulating the chemical reactants and quantitative dynamics.

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 [26]. Sub-acute exposure to MgO showed favorable and non-toxic effect towards oxidative stress. 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 [27]. MgO nanoparticle supplementation in an acute lung injury rat model had a quantifiable and alleviating effect in an acute the lung injury associated systemic disorders. 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 ($P<0.05$). These parameters also showed a dose-dependent effect on the lung injury status with regards to the MgO nanoparticle ($P<0.05$). The overall effect of the HDT-MgO 300 mg/kg nanoparticle administration was greater than the other experimental groups ($P<0.05$).

We found significant increased serum TC, TG, HDL-c LDL-c, in positive control (CS-induced lung injury model) and was alleviated by low and high dose of MgO 300 mg/kg the results are opposite to precious finding as the TC, HDL-C and LDL-C concentration was significantly lowered in pulmonary tuberculosis patients than health individuals [28]. Another study suggested that lover level of LDL-c, HDL-c, TC, apolipoproteins A1 and B (ApoA1 and B) was associated with pulmonary severity in COVID-19 patients and administration of lipid lowering drugs reduces the severity but not applicable to all recruited patients.

Acute Respiratory Distress Syndrome associated with cellular and structural deterioration in organs and organ system induce liver damage through paracrine cytokine action, hypoxemia, toxins and hypoperfusion manifested by increased serum level of AST and ALT [29] finding are inconsistent without results as lung injury associated liver dysfunction exhibit marked increase AST and ALT in CS- lung injury rats, changes were normalized following sub-acute exposure to LDT-MgO 150 mg/kg and HDT-MgO 300 mg/kg.

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 LDT and HDT 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 NPs were selected because their bulk supplemental form has already been explored for toxic 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. Following sub-acute exposure to high dose MgO nanoparticle, significantly increased the level of hemoglobin ($P<0.05$).

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 HDT-MgO 300 mg/kg NPs recovered remarkable and sufficiently normalized the lung injury. It would be productive to investigate the active compounds of the nanoparticle supplementation responsible for prevention and amelioration of lungs tissue damage model rats.

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. 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 [30]. Owing to 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 done far in this area suggested that NP characteristics such as size, surface charge, and hydrophobicity are critical factors for their biological functions [31].

CONCLUSION

Conclusively sub-acute exposure of our selected MgO nanoparticle formulation exhibited dose dependent potential ameliorative effect on acute lung injury and associated hepatic, oxidative stress, lipid profile and hematological dysfunctions. However, the further investigations are required to explore mechanism of their action and diffusion kinetics.

DECLARATIONS

ETHICAL APPROVAL

The study was conducted in Koc University graduate school of health sciences. All methods were performed in accordance with relevant guidelines and regulations of "local ethics committee for animal experiments of Koc university." The study was approved by the committee with Approval No. (2023-5). The rats were kept in the Koc University, Animal Research Facility (KUARF) of Centre for Translational Medicine (KUTTAM). All of animal procedures were carried out under the standard rules established by the Governing Board of the National Research Council, whose members are drawn from the Institute of Medicine. The members of the Committee responsible for the study were chosen for their special competences and with regard for appropriate balance.

AUTHOR CONTRIBUTIONS

AS wrote the manuscript, ASK reviewed and edited the manuscript. All authors have read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during study was presented in article.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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