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# Efficacy study of Ceftriaxone plus Sulbactam with SFI on Oxidative Stress, Hematological and Enzymatic Parameters in Lower Respiratory Tract Induced Rat Model.

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**ABSTRACT**

Problems- Study was conducted to evaluate the combinational use of antibiotics (ceftriaxone + sulbactam) with a solvent (chelating agent) in LRTI infection. Experimental approach - Assay for the free radicals, enzymatic level, and analysis for the hematological were done after induction of disease (7$^{th}$ day) and after 7 day treatment (14$^{th}$ day). Histopathological analysis was done at the end of the study in Sprague Dayle rats. Results were analyzed by One-way (ANOVA) followed by paired t- test. Major findings and conclusion- After the treatment, SOD (p=0.05) and catalase (p=0.05), MDA (p=0.05) and inflammatory (p=0.05 & p=0.05) for IL-6 and TNF- respectively) markers reduced significantly and group cef+sul+SFI showed best recovery, and histopathological parameters (inflammation, neutrophils) improved and group cef+sul+SFI showed better recovery. The novel combination of antibiotics (ceftriaxone and sulbactum) with SFI showed better recovery in LRTI rats.

**Keywords:** Lower respiratory tract infections, *K. pneumoniae*, Solvent for Injection, oxidative stress, Free Radicals.

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## INTRODUCTION

Lower respiratory tract infections (LRTIs) are the most common infectious diseases and important causes of morbidity and mortality among all age groups. Each year approximately 7 million people die by the direct infection of acute and chronic respiratory infections. [1- 2] LRTIs are frequent and include Community-acquired pneumonia (CAP), exacerbation of chronic bronchitis, acute bronchitis and viral lower respiratory tract infection. [3] *Klebsiella pneumoniae* is the most common pathogen that causes lower respiratory tract infections, community acquired, as well as nosocomial lung infections. An increasing risk for developing pneumonia among all age group population, and an increase in the emergence of multidrug resistance among *K. pneumoniae* nosocomial isolates has renewed interest in the investigation of alternative approaches for the treatment. [4- 5] It causes acute inflammation in the lung by increased activity of neutrophils generates oxygen free radical and decreased the endogenous anti-oxidant defense system. [6]

Ceftriaxone is a third generation cephalosporin class of β-lactam antibiotic drug with potent bactericidal activity against a wide range of gram positive and gram negative bacteria due to inhibition of bacterial cell wall synthesis. [7- 8] Sulbactam is a β-lacatamase inhibitor and a cephalosporin class of antibiotic with broad spectrum activity against gram negative organisms and effective in preventing the free radical-mediated oxidation of sulfhydryl groups. [9-10] It has proven that cephalosporins act as multidentate chelating agents. [11] Solvent for injection (SFI) contains Ethylene diamine tetra acetate (EDTA), a third vector with these two drugs. SFI has chelating, antioxidant and free radical scavenger properties. Many published studies confirmed its safety and effectiveness. [12- 13]

This study was aimed to find out the efficacy and protective effect of ceftriaxone plus sulbactam with SFI on hematological, antioxidant enzyme and inflammatory marker in plasma and tissues of Lower Respiratory Tract Infection exposed Sprague dayle rats.

## MATERIALS & METHODS

Chemicals used for this study were obtained from Sigma, St. Louis, MO, USA and analytical grade chemicals were purchased locally. Biochemical kits used for this study were purchased from Bayer Diagnostics India Ltd., Baroda, Gujrat, India. The study was approved by the institutional animal ethical committee (IAEC) of Venus Remedies Pvt. Ltd. The Sprague Dayle (SD) rats (weight 65-105 gm) were obtained from animal house facility of Venus Medicine Research Centre, Baddi, Himachal Pradesh, India and kept a temperature (23 ± 2°C) and humidity (65 ± 5%) controlled room. Culture medium of *Klebsiella pneumoniae* organisms were obtained from IMTECH (Institute of Microbial Technology), Chandigarh, India.

In this study, thirty male SD rats were divided into five groups (six rats in each group): - I: Control group, II: Control normal saline treated group (0.5 ml NaCl), III: *K. pneumoniae* infected (50µl log $10^6$ colony-forming units (CFU)/ml) group, IV: Infected +ceftriaxone plus sulbactam treated group (155 mg/Kg body weight/bid) and V: Infected + ceftriaxone plus sulbactam + SFI treated group (186 mg/Kg body weight/bid). After pre and post treatment blood samples were collected for estimation of blood parameters. Rats were sacrificed at the end of experiment, lung tissues were removed aseptically and lung tissue photograph were taken immediately for gross observation changes in each group. Lung tissue was sectioned into two halves, one half of lung was homogenized in 1ml normal saline and histological examination was done in other half part of lung tissue.

**Experimental:**

**Induction of Lower respiratory tract infection**

A single isolated colony of bacterial strain were maintained on nutrient agar slant, nutrient agar plate was transferred to 50 ml nutrient broth and incubated at 37°C for 18 h. Organism were harvested by centrifuged at 2500 x for 20 minutes and washed to 3-4 times with sterile PBS (0.2 M, pH 7.2).

For induction of lower respiratory tract infection in rat model, bacterial dose 50µl of log $10^6$ CFU/ml and the method of Held et al was employed for intranasal instillation of the bacterial inoculums. [14] For induction of LRTI, bacterial dose was instilled into the nasal opening while holding the rat upright for 7 days

twice daily (at 10.00 a.m. and 5.00 p.m.). Total 18 rats (six rats in each group) were infected and confirmed by increased body temperature, sneezing, coughing, labored movement (Walking with difficulties), cell count (WBC) and presence of bacterial count in blood sample. From remaining 12 rats, six were neither infected nor treated (Group I), while six were treated with 0.9% NaCl only (Group II).

**Treatment of infection**

Antibiotics ceftriaxone (1000 mg) and sulbactum (1500 mg) and SFI were obtained from Venus Remedies, India. After confirmation of disease, ceftriaxone + sulbactum and ceftriaxone + sulbactum + SFI drugs were given via intravenous route for 7 days. The ratio of fixed dose combination of ceftriaxone + sulbactum and SFI was 1: 2 respectively.

**Preparation of Plasma and Lung homogenate**

Blood samples were collected by retro orbital vein in sodium citrate (3.8%) containing vial and centrifuged at 0-4◦C at 6000 rpm for 15 minutes and plasma was collected for determine enzyme activity along with malonaldialdehyde level and biochemical parameters of a ll groups. Lung tissue homogenates (10%) were prepared in chilled phosphate buffer-NaCl solution containing 0.15 mol/L NaCl in 0.05 mol/L, Na2HPO4-NaH2PO4 buffer (pH 7.2) and left for at least 1 hr at 2–8°C before measurement of MDA.

**Determination of Hematological and Biochemical parameter**

The automatic cell counter (Sysmex XT 2000i) was used for the determination of RBC, hemoglobin, WBC and platelets levels. The biochemical parameters (albumin, total protein, triglycerides) were measured by fully automatic biochemical analyser (Erba Manheim; Model EM200) in all groups.

**Superoxide Dismutase (SOD) assay**

In plasma SOD assay was determined by the Method of Misra and Fradovich. [15] The assay consisted of 1.0 ml carbonate buffer (0.2 M, pH 10.2), 0.8 ml KCl (0.015 M), suitable aliquot of lung tissue homogenate and distilled water to make the final volume to 3.0 ml. The assay started by adding 0.2 ml of epinephrine (0.025 M). Absorbance was recorded at 480 nm at 15 second interval for one minute at 25◦C. A preparation of control lacking enzyme was run simultaneously. The amount of enzyme causing 50% inhibition of auto oxidation of epinephrine defines one unit of enzyme activity.

**Catalase assay**

In plasma Catalase activity assay was measured by the method of Luck. [16] The assay consisted of 0.3 ml phosphate buffer, (0.2 M; pH 6.8), 0.1ml $H_2O_2$ (1 M) and distilled water to make the final volume to 3.0 ml. The assay was started by adding the suitable aliquot of lung homogenate. The absorbance was recorded at 15 sec interval for one minute at 240 nm at 25◦C. Suitable control was run simultaneously. One Unit of enzyme activity was defined as the amount of enzyme that liberates half of the peroxide oxygen from $H_2O_2$ in 100 sec at 25◦C.

**Malonaldialdehyde (MDA)**

Lipid peroxidation measurement to determine the extent of lung tissue damage was assessed by the measurement of malonaldialdehyde (MDA) according to Ohkawa et al. [17] The reaction mixture consisted of 0.20 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of (20%, pH 3.5) acetic acid, suitable amount of homogenate preparation, 1.5 ml of 0.8% thiobarbituric acid (TBA) and distilled water to make up the volume to 4.0 ml. This mixture was then kept in a boiling water bath for 1 hour and cooled immediately under running tap water. Added 1.0 ml of water and 5.0 ml of mixture of n-butanol and pyridine (15:1 v/v) and shacked vigorously and centrifuged at 3500 rpm for 30 minutes. The upper organic layer was taken in a separate tube and optical density was measured at 532nm. The reference standard was used 1,1,3,3 tetraethoxy propane for calculation and concentration was expressed as µ mol/ mg protein.

**Cytokines assay**

Serum cytokines TNF-α and IL-6 were assayed using invitrogen kits (Camarillo, CA, USA) following manufacturer's instructions.

**Histopathological analysis of Lung tissue**

Lungs were removed aseptically and collected in 10% buffered formalin saline for histological studies. The formalin fixed tissues were washed overnight in running tap water and dehydrated in ascending series of alcohol (70-100%) and cleared in benzene. For routine histopathology, the 4-5 micron thick tissue sections were cut from the paraffin embedded tissues and was stained with haematoxylin and eosin stain (H&E) (Luna 1968). Morphological changes were assessed by using routine light microscopy.

**Statistical analysis**

All values were presented as Mean ± SD. One-way analysis of variance (ANOVA) followed by paired t-test was used to determine statistical difference between control *vs.* saline treated group and disease induced group and disease induced group *vs.* both treated groups. The p-values <0.05 were considered statistically significant.

## RESULT AND DISCUSSION

In physical parameter we observed body weight, food intake, water intake and temperature at time of 1$^{st}$ day, 7$^{th}$ day and 14$^{th}$ days. We observed that significant changes (p=<0.05) after induction of disease (7$^{th}$ days) in physical parameters as compared to control rats. After administration of a novel fixed combination of ceftriaxone + sulbactum and ceftriaxone + sulbactum with SFI drugs for seven days treatment, body weight, food intake, water intake and temperature were significantly improved as compared to non-treated group. When we compared both treated group with each other's, ceftriaxone + sulbactum with SFI treated group showed better recovery in term of all physical parameters but only food intake parameter was statistically significant (Table 1).

In heamatological parameters we measured RBC, heamoglobin, WBC and platelets at time of pre (7$^{th}$ day) and post treatment (14$^{th}$ day). In diseased group we observed significantly low level of heamoglobin and RBC count, whereas high level of WBC and platelets count but statistically not significant as compared to control group. After treatment with ceftriaxone + sulbactum and ceftriaxone + sulbactum +SFI group, WBC and platelets count was drastically reduced in both treated group as compared to non-treated group. The level of heamoglobin and RBC were increased in treated group as compared to non-treated group but only heamoglobin showed statistically significant increased level. When we compared both treated group with each other's, Ceftriaxone + Sulbactum with SFI treated group showed better recovery in term of RBC, Hemoglobin, WBC and Platelets but none of these were statistically significant (Table 2).

The levels of biochemical parameters (total protein albumin and triglyceride) were increased significantly in diseased group as compared to healthy control group except albumin level. These biochemical parameters were significantly lowered in ceftriaxone+ sulbactum treated group as well as in ceftriaxone+ sulbactum +SFI treated group after seven days of treatment as compared to infected group. When ceftriaxone+ sulbactum +SFI treated group was compared with ceftriaxone+ sulbactum treated group, these biochemical parameters were lowered in ceftriaxone+ sulbactum +SFI treated group after seven days treatment but statistically not significant (Table 3).

Enzymatic and non-enzymatic antioxidant enzymes activities (Superoxide dismutase, Catalase) were significantly decreased in K. *pneumoniae* infected group as compared with control group. These enzyme activities were significantly increased in ceftriaxone+ sulbactum treated group as well as in ceftriaxone+ sulbactum +SFI treated group when compared with infected group after seven days treatment. But in case of ceftriaxone+ sulbactum treated group vs. ceftriaxone+ sulbactum +SFI treated group, only catalase activity significantly (p=0.017) reduced in ceftriaxone+ sulbactum +SFI treated group after seven days treatment. In case of MDA level, in diseased condition it increases significantly and after treatment level was significantly decreased in treated group (Table 3).

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**Table 1**: **Effect of ceftriaxone plus sulbactam with Solvent for Injection on Physical Parameters in Lower Respiratory Tract Infection Induced Rat Model.**

| Physical Parameters | Days | Control Group (N=6) | Saline Treated (N=6) | Disease Control (N=6) | Cef + Sul (N=6) | Cef + Sul + SFI (N=6) |
|---|---|---|---|---|---|---|
| **Body Weight** (gram) | 1 | 78 ± 18.23 | 85 ± 15.32* | 98.83±15.91* | 103±9.77* | 100±10.48* |
| | 7 | 79.5 ±18.7 | 85.8±11.92* | 82.3 ±13.89* | 83.5±8.04* | 79±10.11* |
| | 14 | 81.3± 15.64 | 86.6± 9.95* | 61.3±13.67** | 84.8±3.60** | 88 ±7.61** |
| **Food Intake** (gram) | 1 | 37.8±10.43 | 37±11.31* | 37.3±10.11* | 40.1±7.38* | 34.1±6.96* |
| | 7 | 36.8±3.65 | 41.3±5.88* | 21± 5.89** | 15.3±2.94* | 17.3±2.16* |
| | 14 | 39.5 ±0 .83 | 42.6±2.87* | 10.1±1.47** | 25.5 ±2.58** | 31 ±3.09** |
| **Water Intake** (ml) | 1 | 34 ±3.84 | 33.8±7.11* | 17.5± 2.94* | 29 ±4* | 29± 3.8* |
| | 7 | 33.3±3.93 | 32 ± 8.94 | 18.5 ± 3.88** | 18.6±2.42* | 19.5±3.20* |
| | 14 | 38.8±2.31 | 38.1± 3.48* | 10.8± 1.47** | 27.8±3.06** | 32 ±2.52** |
| **Temperature** (◦C) | 1 | 34.4 ±3.0 | 33.2± 3.50* | 32.9± 3.37* | 36.0±3.22* | 36.9±1.44* |
| | 7 | 34.9±1.00 | 34.65±1.51* | 42.55± 0.96** | 42.7±1.26* | 42.9± 0.81* |
| | 14 | 34.35 ±1.34 | 33.3± 1.30* | 43.65 ±0.37** | 36.8± 0.85** | 35.4 ±1.06** |

N=Number, Data presented as mean ± SD. Analysis was done between control vs. disease control and saline treated as well as disease control vs. Cef + Sul and disease control vs. Cef + Sul + SFI and Cef + Sul vs. Cef + Sul + SFI. Where * = p >0.01; not significant=** p <0.05 significant. N=Number, Data presented as mean ± SD.

**Table 2: Effect of Ceftriaxone plus Sulbactam with Solvent for Injection on Heamatological Parameters in Lower Respiratory Tract Infection Induced Rat Model.**

| Hematological Parameters | Duration | Control Group (N=6) | Saline treated (N=6) | Disease Control (N=6) | Cef + Sul (N=6) | Cef + Sul + SFI (N=6) |
|---|---|---|---|---|---|---|
| RBC ($10X10^6/\mu l$) | Pre | 7.7 ±1.0 | 7.90±1.24* | 5.49 ± 0.97** | 4.8 ± 0.817* | 4.5 ±.50* |
| | Post | 7.9± 1.14 | 7.8± 0.80* | 4.2± 1.15** | 5.57 ±0.78* | 5.83± 0.61* |
| Heamoglobin (g/dl) | Pre | 13.4± 1.79 | 13.6± 1.53* | 9.2± 1.43** | 8.9± 1.34* | 8.5± 1.33* |
| | Post | 13.8 ± 1.24 | 13.6 ± 0.77* | 7.1± 1.51** | 10.2± 1.06** | 10.4 ± 0.77** |
| WBC ($10X10^3/\mu l$) | Pre | 9.5± 2.11 | 9.5± 2.62* | 11.2± 1.50* | 12.9± 0.82** | 11.7± 0.51* |
| | Post | 9.6± 1.72 | 9.6± 1.96* | 14.2± 1.71* | 10.3± 0.83** | 9.8 ±0.56** |
| Platelets (10X3/µl) | Pre | 252.3± 73.03 | 252.3± 73.08* | 259.1± 50.63* | 286.5 ± 92.4* | 380 ± 73.55* |
| | Post | 300 ±70.14 | 300 ±70.16* | 425.3± 39.22** | 168.3±74.54** | 185.8± 65.30** |

N=Number, Data presented as mean ± SD. Analysis was done between control vs. disease control and saline treated as well as disease control vs. Cef + Sul and disease control vs. Cef + Sul + SFI and Cef + Sul vs. Cef + Sul + SFI. Where * = p >0.01; not significant=** p <0.05 significant. N=Number, Data presented as mean ± SD.

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**Table 3: Effect of Ceftriaxone plus Sulbactam with Solvent for Injection on Biochemical Parameters and Enzymatic Parameters in Lower Respiratory Tract Infection Induced Rat Model.**

| Biochemical Parameters | Duration | Healthy Control (N=6) | Saline treated (N=6) | Disease control (N=6) | Cef + Sul (N=6) | Cef + Sul + SFI (N=6) |
|---|---|---|---|---|---|---|
| **Total Protein** (g/DL) | Pre | 6.6 ±0 .56 | 6.6 ± 0.45* | 8.75± 1.66* | 9.1± 1.34* | 8.4 ±1.18* |
| | Post | 6.6± 0.45 | 7.0 ± 0.74* | 13.2± 2.66** | 7.2±0.92** | 6.1± 0.56** |
| **Albumin** (g/DL) | Pre | 4.2 ±0.45 | 4.3± 0.63* | 5.8± 1.33* | 8.05 ±1.08* | 8.1± 1.12** |
| | Post | 4.3 ± 0.31 | 4.5± 0.45* | 8.13± 1.34** | 6.6± 1.12* | 6.1± 0.68** |
| **Triglyceride** (mg/DL) | Pre | 65.6±0.91 | 62.8± 1.51* | 76±6.57** | 76.8 ±6.74* | 78.6±7.38* |
| | Post | 70± 4.14 | 69. ±3.79* | 93.7± 13.30** | 69.2 ± 7.74* | 67.8± 7.57** |
| **SOD** (mg/DL) | Pre | 0.6±0.039 | 0.59± 0.073* | 0.40± 0.059** | 0.47± 0.110* | 0.46± 0.067* |
| | Post | 0.62± 0.030 | 0.58 ± 0.092* | 0.25± 0.084** | 0.63±0.111** | 0.71± 0.142** |
| **Catalase** (mg/DL) | Pre | 0.21± 0.003 | 0.20 ± 0.009* | 0.16± 0.015** | 0.16± 010* | 0.17 ± 0.039* |
| | Post | 0.21± 0.002 | 0.21± 0.003* | 0.134± 0.009** | 0.17± 0.018* | 0.22 ±0.054** |
| **MDA** (mg/DL) | Pre | 0.08 ±0.0118 | 0.07± 0.011* | 0.039± 0.044* | 0.039±0.043* | 0.037 ± 0.039* |
| | Post | 0.08 ±0.011 | 0.07± 0 .015* | 0.012±0.0009** | 0.072±0.031** | 0.066±0.031** |
| **TNF-α** (pg/Ml) | Pre | 331.67 ± 8.89 | 333.3± 7.35* | 423.3 ± 69* | 418.5± 73.44* | 414.3± 71.99* |
| | Post | 332.45 ± 6.30 | 334.5± 7.80* | 557.9 ± 89.5** | 350.9±84.88** | 292.9 ±57.8** |
| **IL-6** (pg/Ml) | Pre | 429.3± 21.93 | 429.7±16.36* | 500.6± 37.05** | 478.9± 40.99* | 481.8± 47.86* |
| | Post | 437.8± 17.52 | 439± 19.30* | 638.7±46.037** | 391.1±50.32** | 318.5±45.75** |

N=Number, Data presented as mean ± SD. Analysis was done between control vs. disease control and saline treated as well as disease control vs. Cef + Sul and disease control vs. Cef + Sul + SFI and Cef + Sul vs. Cef + Sul + SFI. Where * = p >0.01; not significant=** p <0.05 significant. N=Number, Data presented as mean ± SD.

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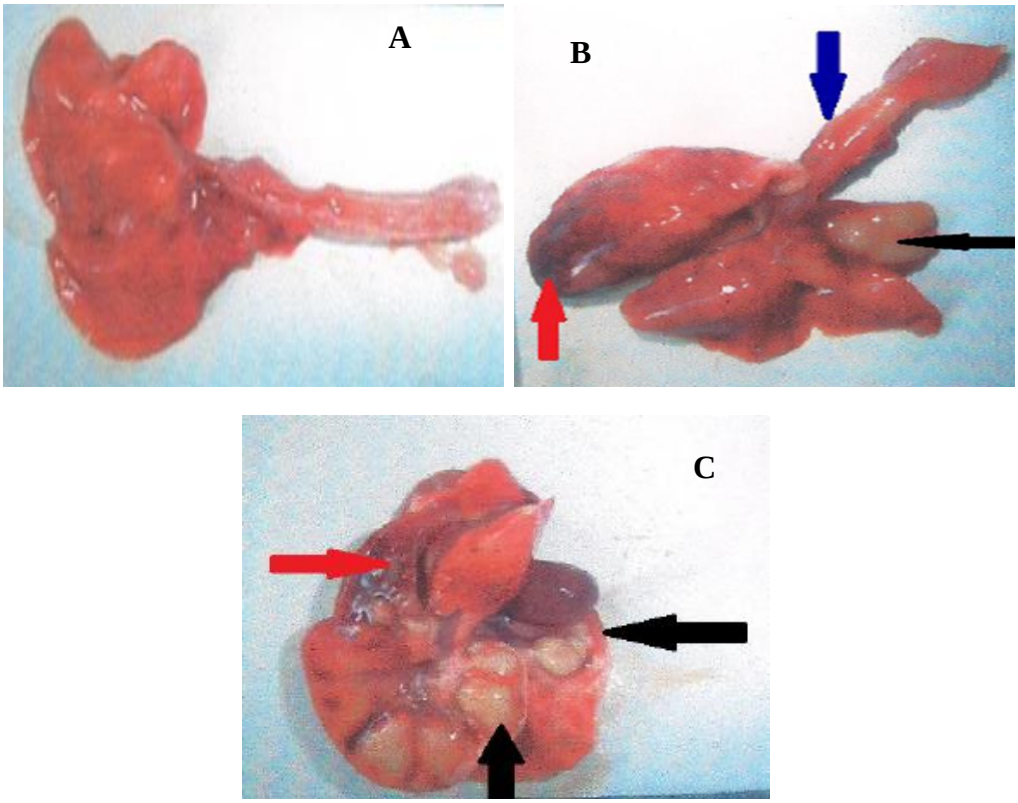


**Figure 1: Morphological observation of lung isolated from Lower Respiratory Tract of rat.**

**A**: Healthy rat, **B and C**: Infection induced rats (Red arrow shows tissue necrosis due to neutrophilic infiltrations and bacterial infection, black arrow shows pus formation in lungs alveoli and blue arrow shows inflammation in trachea due to cytokines and other inflammatory mediators).

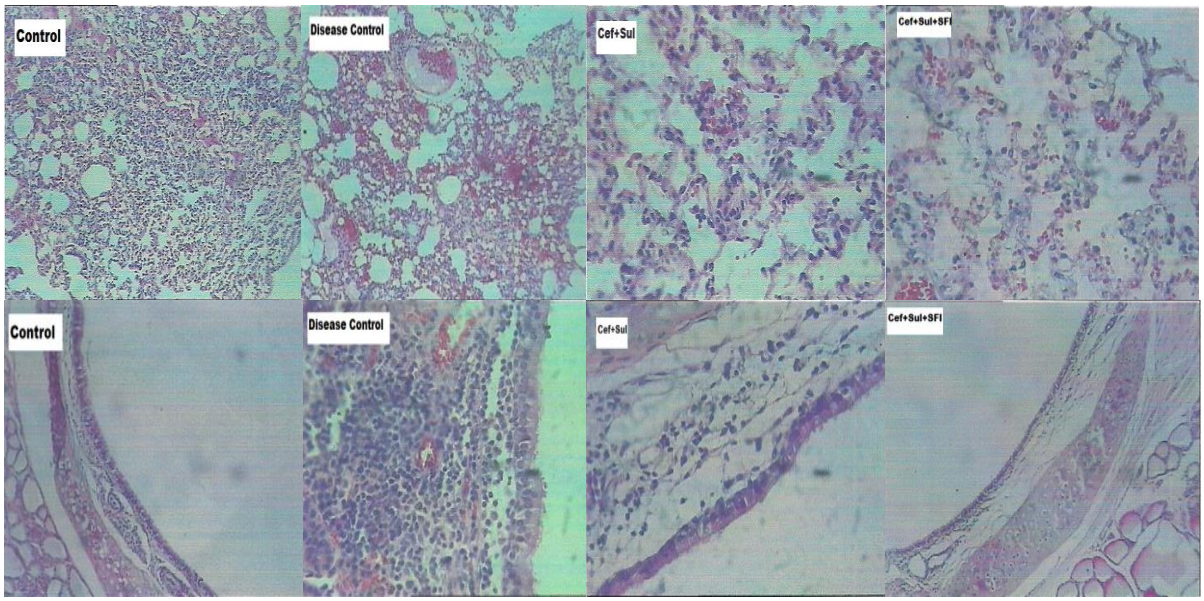


**Figure 2. Microscopic histopathology of different groups of studied rats of Lung (up) and Trachea (below).**

In **control group (Figure 2A) -** Lungs and trachea showed normal histological picture in control group, in **Disease group (Figure 2B) -**Lung sections showed edema formation along with hemorrhages and congestion. The tracheal section showed severe lymphocytic infiltration and mucous epithelial erosion**. Cef+Sul Treated group (Figure 2C) -**Lung section showed mild focal emphysematous changes were seen microscopically. The tracheal section showed mild lymphocytic infiltration**.Cef+Sul+SFI treated (Figure 2D)** -Lung section showed mild congestion. The tracheal section showed near normal histological picture with few lymphocytic infiltrations.

In diseased condition, cytokines IL-6 and TNF-α both significantly increased as compared to healthy rats. The levels of both cytokines were significantly lowered after seven days treatment with ceftriaxone + sulbactum and ceftriaxone + sulbactum + SFI. When we compared both treated groups with each other, ceftriaxone + sulbactum + SFI treated rats showed significantly lower level of IL-6 (Table 3).

In morphological study of lungs and trachea, lung and trachea showed normal colour and normal morphological structure in control group (Fig. 1A). The gross examination of respiratory tract of lower respiratory infection induced rats (red arrow) shows tissue necrosis due to neutrophilic infiltrations and bacterial infection (black arrow) shows pus formation in lungs alveoli (blue arrow) shows inflammation in trachea due to cytokines and other inflammatory mediators in lower respiratory infection (Fig. 1B-1C).

In histopathological study, the microscopic examination of lungs and trachea showed normal histological picture in control group (Fig. 2A). In infected group, lung sections showed edema formation along with hemorrhages and congestion and tracheal section showed severe lymphocytic infiltration and mucous epithelial erosion (Fig. 2B).

In ceftriaxone + sulbactum treated group, mild focal emphysematous changes were seen microscopically and tracheal section showed mild lymphocytic infiltration (Figure 2C). The ceftriaxone + sulbactum + SFI treated group showed mild congestion were seen in the lung tissue and tracheal section showed almost normal histological picture with few lymphocytic infiltrations (Figure 2D)

## DISCUSSION

Lower respiratory tract infections (LTRIs) are one of the challenging and severe forms of infections in front of health care practitioners .[18] *K. pneumoniae* was the most predominant (25-43%) isolate recovered from patients with LRTIs and nosocomial pneumonias and it is a common pathogen that causes community-acquired pneumonia and nosocomial infections . [19-21] *K. pneumoniae* causes acute inflammation in the lungs when infected by intranasal route and increases neutrophil activities with increased oxidative stress and various inflammatory biomarkers. In this study, we observed the role of the malondialdehyde (MDA) in tissue, tumour necrosis factor (TNF)-*α* and IL-6 in the serum. These parameters were increased in *Klebsiella pneumoniae* induced groups as compared with control group but only IL-6 increased significantly (Table 3). MDA and cytokines levels were increased due to presence of *Klebsiella pneumoniae* in the blood (TNF-α and interlukin-6) and in lung tissue (MDA) of LRTI induced group compare to control group.

Phagocytes cell are activated when the respiratory infections are happens by the distal airways, whenever these phagocytes cells uncounted bacteria, these cell releases excess free radicals as a host defense against infection. Per oxidative lipid damage occurred whenever reactive oxygen free radicals are produced, so this damage may be reduced by antioxidant enzymes. Superoxide dismutase (SOD) and catalase are free radical scavenging enzymes that inhibits the generation of free radicals. [22] In this study we observed SOD and catalase enzymes activities were significantly decreased in infected group as compared with control group (Table 3).

Oxidative stress is found increased in various types of respiratory disorders. The condition of asthma, chronic obstructive pulmonary disease and bronchiectasis causes inflammation and increased levels of oxidative stress. [23] Many studies showed that *Klebsiella pneumoniae* causes oxidative stress in respiratory infection. [24] During *Klebsiella pneumonia* infection, the levels of antioxidant can be decreased due to generation of oxidative stress in the lung tissue. In this study, the albumin, total protein and triglyceride levels were also elevated in the LRTIs induced group. The increased levels of albumin and total protein and triglyceride showed that bacterial infection in lung tissue caused inflammation and cytotoxicity. Many studies showed that due to *Klebsiella pneumoniae* infection the level of protein and albumin increased in rat model. [25]

Antibiotic used for LRTIs remains controversial. In a case of LRTI, the exclusion of CAP (community acquired pneumonia) in out-patients is very difficult and also antibiotic are used for self-limiting illnesses, so it may cause resistance of bacteria to antibiotics. Because LRTIs is mainly treated by antibiotics, so changes in antibiotic resistance patterns are a threat to the effective treatment of CAP. [26]

It is observed that combination drug therapy is more effective due to their synergistic effect than individual drug therapy. [2-28] ceftriaxone is antibiotic drug with potent bactericidal activity against a wide range of gram positive and gram negative bacteria [7], and sulbactam is a β-lacatamase inhibitor with better broad spectrum activity against gram negative organisms.[9] While SFI (solvent for injection) is Ethylene diamine tetra acetate (EDTA) a third vector with these two drugs. SFI has chelating, antioxidant and free radical scavenger properties [12-13], that act against the microorganism for any of the biological role of metal-dependent proteins which are associated to the maintenance of their transcription factor. Due to the role of metal –dependent protein, it enters into the cell membrane and opens the $Ca^{+2}$ Channel and increased the concentration of combination drug in bacterial cell leading to inhibition of microbial cell transcription and death. The SFI binds with divalent metal ions and make them unavailable for the bacteria witch are essential for cellular replication and growth. [29] In this study ceftriaxone + sulbactum + SFI combination showed better antimicrobial efficacy against gram negative bacteria than ceftriaxone + sulbactum. The levels of catalase ($p=0.01$), cytokine (IL-6) ($p=0.03$) were also significantly decreased in the combination drug of ceftriaxone + sulbactum +SFI treated group than ceftriaxone + sulbactum alone after seven days treatment. In this study we also observed that cef+sul+SFI showed better free radical scavenger property than ceftriaxone + sulbactum combination which reduced lung tissue injury. Many studies have observed that ceftriaxone + sulbactum have neutrophils inhibition function and free radical scavenger property. [30]

## CONCLUSION

To conclude, study showed that a novel combination of **cef+sul**+SFI drugs showed better efficacy in terms of antimicrobial and free radical scavenger property by inhibition of the free radical, which causes tissue injury and inflammatory response in lower respiratory tract infections in SD rats.

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