9.1. INTRODUCTION

*Klebsiella pneumoniae* is a leader to cause nosocomial and community-acquired gram-negative bacterial pneumonia, which spreads through the air media, causes severe pyogenic infection in lungs and urinary tract and exerts high mortality rate in the absence of therapeutic intervention (Podschun., and Ullmann, 1998). The cellular components of the lung are continuously renewed through the life process. When the epithelium of airway was exposed to the many materials such as pollutants, pathogens and other chemical substances through alveolar compartments. These constant exposure to airborne environmental components cause stresses subsequently injury too. Initially they are able to adapt to injury from these airborne diseases such as *K.pneumonia* infections, if fails mortality results. Thus there is a need to develop some new novel antimicrobial agents to overcome such events. The search for new and effective antimicrobial agents to treat bacterial or fungal infections has focused in recent years on components of the innate defense systems of plants and animals. In this array antimicrobial peptide was identified and administered against *K. pneumoniae*.

The histoarchitecture manifested the history of cells; the acute and chronic toxicity leads impairment in the vital tissues (Gunn and Gould, 1970). The acute toxicity leads to organism’s death but the chronic level leads to organ infection that cause severe necrotic and tissue damaging i.e., the slow killing process of organism. The organisms suffer to pathetic and slow all the physiological and metabolic functions (de Kretser, 1974). Infection of the host by bacteria leads to the activation of multiple defense signaling pathways that are critical for the removal of the invading pathogen. These signaling events induce the production of pro inflammatory cytokines and antimicrobial molecules that are important for bacterial clearance (Inohara et al, 2005; Akira et al., 2006). Therefore the present study, aimed to investigates the challenging of *K. pneumoniae* with different treatments such as drug ciprofloxacin, PL-peptide and PL-peptide encapsulated with chitosan Nanoparticle and *K. pneumoniae* alone. The evaluation of changes in Histopathology elicits the action of PL-peptide against *K. pneumoniae* infection in the Challenging mice.
9.2. MATERIALS AND METHODS

The mice target tissues liver and lung were collected and fixed with fixatives for histological analysis. Bouin’s is used because of its capability for rapid fixation (24 hrs) and strong subsequent tissue staining. However, intracellular substances, such as granules and inclusions, are often poorly preserved with this fixative embedding. Embedding of the mice liver space and lung can be accomplished rapidly as follows:

- 30% ethanol 30 min
- 50% ethanol 30 min
- 70% ethanol 30 min
- 90% ethanol 30 min
- 100% ethanol 30 min

Lung and liver are embedded in the same longitudinal orientation as with paraffin blocks. They are also sectioned in the same step-wise manner as paraffin blocks, but at a thickness of 2–3 µm. Staining of methacrylate sections can be accomplished as with paraffin procedures, but with slight modifications.

9.1.1. Sectioning and staining.

Standard sectioning and staining procedures can be used with fathead minnow tissues. For example, sections may be cut at 5 µm and stained with hematoxylin and eosin. For lungs and livers, typically in the laboratory, a number of slides are made, each with one section from 500µm deep into the longitudinally oriented the organ.

**Hematoxylin and eosin (with phloxin)**

- Stain sections for 30–45 min with filtered Harris hematoxylin
- Rinse with distilled water
- Dry on a hot plate
- Stain cooled slides for 1–2 min in saturated aqueous eosin containing 0.25% phloxin
- Rinse in distilled water, dry on a hot plate, and coverslip

**Basic fuschin and methylene blue–azure A**

- Stock basic fuschin:
  - 1% basic fuchsine in 50% ethanol
- Stock methylene blue–azure A in distilled water
  - 1% azure A
  - 1% methylene blue
  - 1% borax
Dilute basic fuchsin 1:4–1:12 or more with distilled water
Stain 10–20 s and rinse with distilled water, dilute methylene blue–azure A1:2–1:4 or more with distilled water Stain 10–20 s, rinse with distilled water, dry, and coverslip

9.3. RESULT

The control liver and lung architecture was no remarkable changes whereas the all the treated groups are remarkably changed in both organs. In liver hepatocytes and clear alveolar sac with normal thinned alveolar layer, air spaces and blood vessels.

9.3.1. The drug with *K. pneumoniae* group

**Liver**

The drug with *K. pneumoniae* groups liver Photo-1 showed liver, medial fibrinoid degeneration with periarterial inflammation was frequently observed in hilar arteries. During 12 hr, they started the liver cell degeneration; in 24 hr duration the section shown red cell rigidity and hepatic fibrosis occurred. In the case of 36 hr duration shown portal inflammation and lobule inflammation occurred. Whereas the 48hr duration shown gradual reduction of inflamed lobules and during 72 hr the tissues was gradually restored with mild steatosis occurred.

**Lungs**

During 12 hr the alveolus had degenerated and portal inflammation occurred, in 24 hr duration severe interstitial edema hemorrhage and inflammatory cell infiltrate occurred also shown red cell filled in the air sacs. In the case of 36 hr duration necropsy of lung epithelial cells, diffuse infiltrate cell inflammatory and fibrosis lung tissues. Whereas the 48hr duration, the tissues was shown the initiation of regeneration with less cell infiltrate finally during 72 hr duration has been shown gradual recovered alveolus.
9.2.2. Histology of liver and lung experimented with Pleurocidin like peptide and *K. pneumoniae* treated mice.

**Liver**

In 12 hr duration, the initiation of hemorrhage occurred in the hepatic cells, in 24 hr duration shown portal inflammation and hepatocytes degeneration, in 36 hr duration the portal track lobule are inflamed. In the case of 48hr duration lobular inflammation occurred in the tissues whereas the 72 hr duration the portal tract reappeared.
Photo-2. Histopathology of *K. pneumoniae* treated mice liver

**Lung**

In lung, thickened alveolar walls noted in 12 hr duration, in 24 hr duration the lung tissues became to change hemorrhage and inflammatory cell infiltrate. In the case of 36 hr duration the section showed the necrosis of alveolar walls and the hemosiderin laden macrophages during 48hr duration. Whereas the 72 hr duration the cell initiated to recover the conditions.
9.3.3. Histopathology of chitoson nanoparticle encapsulated PL-peptide with *K. pneumoniae* treated mice

Liver

During 24 hr the hepatocytes initiated the necrosis then they started parenchymal inflammation with red cell accumulation on the cell space (24 hr). Steatosis and necrosis occurs and seen in number of laden macrophages (36 hr). During
48 hr duration the cell became the initiation of recovered condition; the necrotic conditions were decreased and were seen in less macrophage. In the case of 72 hr duration less steatosis condition occurred in the hepatocytes.

**Photo- 4. Histopathology of mice liver treated with PL-Peptide encapsulated Chitosan nanoparticle**
Lung

Alveolar septal thickened and prominent hyaline membranes were seen also with congested alveolus shown in 12 hr. In the presence of red blood cells in the airspaces and alveolar septum contain pneumocytes proliferation during 24 hr duration. During 36 hr duration blood cell leakage and necropsy occurred. Accumulation of red blood cells and proteinaceous material in inside of the alveolar spaces in 48hr duration whereas the 72 hr duration of cell morphology initiates recovery.

Photo- 5. Histopathology of mice lung treated with Pl-Peptide encapsulated Chitosan nanoparticle
9.3.4. Histopathology of *K. pneumoniae* treated mice

**Liver**

Inflammatory responses within the parenchyma of the liver cells were seen in 12 hr duration in 24 hr duration was shown in lympho- monocyte infiltrations around the portal vein. During 36 hr duration the red blood cells accumulated inside of the hepatic lumen. In continuation of the necrosis became necropsy of hepatocytes during 48 and 72 hr duration.

**Photo- 6. Histopathology of *K. pneumonia* treated mice liver**

**Lung**

The degeneration of alveolus and portal inflammation occurred during 12 hr treated lung, in 24 hr duration the lobule is inflamed. During 24 hr duration the section showed initiation necrosis alveolus in 24 hr duration. Most of the alveolar walls are
damaged and vessel necrosis happened by severe necrosis also cell infiltrate were seen in 36 hr duration of the treated mice. The antracosis pigments were present in this condition during 36 hr duration. In continuation of massive necrosis in the lung alveolus (48 hr duration) and acute inflammatory and cell infiltrate occurred during 72 hr.

**Photo- 7. Histopathology of *K. pneumoniae* treated mice lung**
9.3. DISCUSSION

In each animal, the degree of liver damage was determined in at least five different lobar regions and graded using the modified Suzuki scoring system. Briefly, the various changes noted are sinusoidal congestion, hepatocyte necrosis and ballooning degeneration. The necrosis started at earlier to 36 hr or 48hr in all the treated groups except *K. pneumoniae* alone, the centrilobular ballooning was started during 12 hrs then gradually severe the congestion/ballooning degeneration as well as >60% lobular necrosis was seen up to 48hr. The drug with *K. pneumoniae* shown the initial recovery started during 36 hr duration whereas the chitosanic nano encapsulated PLP with *K. pneumoniae* groups were shown the recovery period was started at 48h duration. The remaining group of PLP with *K. pneumoniae* was shown in the same category but comparatively slow process. The *K. pneumoniae* treated mice shown severe necrosis and established their colonies in the liver tissues. That seems the cause for severe necrosis and lethality. Similar process occurred in the *E.coli* treated mice (Kasman, 2005). The liver and lung inflammation occurred (Lawlor et al., 2005). *K. pneumoniae* can be isolated from the upper respiratory and GI-tracts of both man and mouse and thus is considered to be a normal component of the microbiota at these mucosal sites (Ostfeld et al., 1983; Cangemiet al., 1999; Kasman.2005). Normally, the lower airway (lungs) is kept sterile due to effective host defense mechanisms. Deposition of *K. pneumoniae* into the lower airspace can result in a severe pyogenic infection with high mortality rates without therapeutic intervention. Thus, *K. pneumonia* is a considered to be a clinically significant opportunistic pathogen and is a leading cause of nosocomial and community-acquired gram-negative bacterial pneumonia.

Lungs: Lung interstitial damage ranged from normal to varying degrees of septal thickening, hypercellularity, neutrophilic recruitment, interstitial adhesion and alveolar luminal reduction. The differences in pulmonary epithelial cell responses are dramatic even at an early 12-h time point (Lawlor et al., 2005) in the case of wild type *K. pneumoniae* treated groups. The exact pathological processes of *K. pneumoniae* infection are completely understood. Following an exposure period of 0–72 hr of *K. pneumoniae* manifestations. However, as many as 85% of the infected individuals develop a chronic infection, frequently with severe long-term liver pathology (Hoofnagle, 1997; Chan et al., 2001; Gasparotto et al., 2002; Ishikawa et al., 2003). Chronic *K. pneumoniae* infection has been treated with interferon-α but less than 20%
of patients achieve a sustained response with this drug. (Gretch et al., 1996; Davis et al., 1998; Poynard et al., 1998). The same process was occurred in the present experiment i.e., *K. pneumoniae* with the integration of drug and fish microbial defense peptide treated groups shown different way of histopathological conclusion. So the present study infer the *K. pneumoniae* with drug, chitosanic nanoparticle encapsulated fish defense peptide and fish defense peptide treated groups shown recovered conditions after treatment of 36 hr and 48 hr duration. Whereas the *K. pneumoniae* infection show severe necrosis up to their end of experiment. Among the three treated groups, recovery was similar in drug with *K. pneumoniae* and chitosan encapsulated peptide with *K. pneumoniae*. PLP peptide along with *K. pneumoniae* treated group showed moderate recovery. But the *K. pneumoniae* alone treated groups showed gradual and slow recovery by the histology, therefore the present study concludes that the nano encapsulated PL-peptide treated group suggest the enhancement of chitosan act as best treatment to *K. pneumoniae* infection.