HISTOLOGICAL STUDY OF THE EFFECT OF PSEUDOMONAS AERUGINOSA THAT ISOLATED FROM SAWA LACKING SOME ORGANS IN LOCAL DOMESTIC IRAQI RABBITS (ORYCTOLAGUS CUNICULUS)

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ABSTRACT

The study aimed to estimate the main histological changes in the deferent organs in local domestic rabbits after infected with Pseudomonas aeruginosa after isolated from Sawa lack. The experimental animals were divided into two main groups, a group that considers the treated group that treated with Pseudomonas aeruginosa. The B group which consider as a control group. The histological results after infected in deferent organs were very prominent in all infected organs. The histological results of the lung showed acute degeneration in the tissue structures of lung, there was prominent degeneration in the wall of alveoli. The result showed disappeared the normal tissue structures of the lung. The tissue section of the liver after infected with Pseudomonas aeruginosa showed the liver parenchyma has blood congestions, the hepatic cords were randomly distributions around the central vein. The tissue section showed high aggregations of kupffer cells a rounded the abnormal hepatic cords. The cortical region of the kidney characterized some features which included the abnormal shape of the renal corpuscle, the renal tubules have irregular wide lumen filled with infiltrate and noted Red Blood Corpuscles. The tissue section showed blood congestion between the intestinal villi of the small intestine and disappeared from the basement membrane under the epithelial layer. These results were shown that the Production of extracellular proteases increases the organism's virulence by assisting in bacterial adherence and invasion.

Keywords: kidney, lung, liver, injection

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INTRODUCTION

Pseudomonas aeruginosa is a motile Gram-negative rod-shaped bacterium. It is a highly versatile and adaptable organism that is able to grow, survive and persist under a broad range of environmental conditions and can be found in most of the natural environments including terrestrial, marine and freshwater habitats. Pseudomonas aeruginosa is an opportunistic pathogen that causes extensive morbidity and mortality in individuals who are immunocompromised or have underlying medical conditions such as urinary
tract, respiratory tract and skin infections and primarily causes of nosocomial infections, and it is frequently resistant to commonly used antibiotics and disinfectants.

Microorganisms producing different types of products through the metabolism of various types of substrates. Some of these products considered as toxins that are naturally produced as final products of metabolism not as intermediate, these toxins may be accumulated inside the cell that known as endotoxin or released to growth media known as an exotoxin, these toxins can either be small peptide molecules or proteins. *Pseudomonas aeruginosa* produces large numbers of extracellular toxins, which include phytotoxic factor, pigments, hydrocyanic acid, proteolytic enzymes, phospholipase, enterotoxin, an exotoxin, and slime.

The most important factor in the pathogenicity of *P. aeruginosa* is the elaboration of a group of exotoxins (protein in nature). These exotoxins could cause leukopenia, acidosis, circulatory collapse, necrosis of the liver, pulmonary edema, hemorrhage, and tubular necrosis of kidneys.

Moreover, *P. aeruginosa* causes infections in a wide range of eukaryotic organisms including plants, invertebrates, and vertebrates rendering it an important opportunistic pathogen. While it rarely infects healthy individuals, it is the most common Gram-negative bacterium involved in nosocomial infections of immunodeficient patients. Infections of *P. aeruginosa* can either be acute or chronic. Acute infections such as ventilator-associated pneumonia and urinary tract infections are characterized by rapid bacterial growth, eventually followed by sepsis, and if untreated, frequent death of the host. In contrast, during chronic infections, the bacteria persist within the host for years, e.g. in chronic cystic fibrosis lung infections.

**MATERIALS AND METHODS**

The water samples were collected from several stations of the lake water, where 1 liter sealed glass bottles were used and heated at 140 °C for three hours. The bottle is kept from the bottom so that its mouth is opposite the current (water movement) and opens under water to fill with a small volume Of the bottle, taking into consideration the location of the sample withdrawal to be from the surface water of the lake and to three replicates for each site under study, and then close the bottle tightly, and then keep the samples in a box containing ice until the delivery to the laboratory and start work, and if not work directly The samples are kept at a tetra temperature H 4° for 24 hours, taking into account the samples not exposed to light to keep them from changes in the.

In order to isolate and diagnose the bacteria, the water samples were implanted into the brain infusion broth to activate the bacteria. This medium was an enriched medium and incubated for 24 hours at a temperature of 37 °C. After the incubation for 24 hours and 37°C, a second transplant was carried out for developing colonies on an electrophoresis medium. (Selective media) CHROMAGAR Pseudomonas This medium is a special medium for isolating and diagnosing these bacteria, and they are distinguished by their appearance in blue-green. Bacterial isolation was identified by Bergy's manual.

After obtaining pure bacterial isolation, *Pseudomonas aeruginosa* is harvested in a liquid feed medium (B.H.I. broth) and incubated for two hours at a temperature of 37 °C so that a bacterial bond can be formed to inject it into laboratory animals and to read the results of histological variables. The present work was carried out fifteen adult indigenous rabbits were housed in the lab. House of the college of science under the lab. Environment conditions. The experimental animals were divided into two main groups, one group...
composed of five animals as a control group and other as infection groups which injected intra-cardiac with *Pseudomonas aeruginosa*. The infection of animals dead after thirty minutes of bacterial injection.

**Tissue samples collection:**

The tissue samples collected from infection animals after noted the clinical sings which included (fever, watery diarrhea, and acute nervous sings), the tissue samples were collected from the following organs (lung, kidney, liver, and small intestine). The tissue samples were passed through the deferent steps of histological technique washing with normal saline, fixation with 10% formalin, dehydration with alcohol, clearing with xylene, embedding with paraffin wax, cutting and staining with H&E.

**RESULTS AND DISCUSSION**

**Lung:**

The results of the control group noted the tissue section of the lung has pneumocyte with irregular nuclei. The lung has different branches of the bronchial tree line by respiratory epithelia. The result of the lung appeared to have elastic fibers distributed between the different branches of the bronchial tree. The lower branches of bronchial tree-lined by different types of epithelia started with simple cuboidal epithelia, and in lower branches of the bronchial tree have simple squamous epithelia, (Fig. 1).

![Fig. 1: The tissue section of lung in control group of mice H&E Stain (20x)](image)

For infection lung, the histological results of the lung showed acute degeneration in the tissue structures of lung, there was prominent degeneration in the wall of alveoli. The result showed disappeared the normal tissue structures of the lung. The tissue section showed severe emphysema in different regions of lung. The tissue section noted high inflammatory cell proliferation around the destroyed alveoli, the remained normal alveoli which have large aggregations of pulmonary alveolar macrophages (P.A.M.). These results were maybe because of the high virulence of infected bacteria, the results were agreement with which noted the pathogen lead to Cystic Fibrosis (CF) in patients. The infections with *P. aeruginosa* causes tissues damage (Fig.2). These results agreement with which referred to the main tissue changes because of...
*Pseudomonas aeruginosa* were cystic fibrosis, which effected the lung function. Lung diseases caused by *P. aeruginosa* are a leading cause of death and lead to chronic inflammation, and damaging the epithelial cells' surface.²¹

The tissue section of the lung after injection of the bacterial infection in the endogenous rabbit appeared most bronchial degenerations filled with secretion and exfoliated the epithelial layer and accumulation in the lumen of the bronchial tree. The tissue figure of lung noted prominent blood hemorrhage beside the different bronchial tree. The result showed the lumen of lower bronchial degenerations was filled with (P.A.M.), (Fig.3), with RBCs. the tissue result showed wide cystic dilation filled with fluid in different locations of lung parenchyma. These results were similar to who Saied the excessive neutrophil accumulation results in life-threatening diseases, such as acute lung injury, as well as acute respiratory distress syndrome. Understanding host-pathogen interaction is critical for the development of effective therapeutic strategies to control the damage in the lung.²⁰

![Image](http://doi.org/10.36295/ASRO.2020.231035)
**Fig. 3:** The tissue section of lung in infected group which noted high cellular proliferation around the cystic dilation, showed the thick secretion inside some of dilation H&E Stain (20x).

**Liver**

The present result noted the liver parenchyma composed of the normal hepatocyte. The cells in the liver parenchyma characterized by have acidophilic cytoplasm and prominent nuclei centrally location. The liver composed of many lobules that consider the structural unit of the liver. (Fig.4).

**Fig. 4:** The tissue section of liver in control group which noted normal histological structures of liver parenchyma with normal portal area H&E Stain (20x).

The tissue section of the liver after infected with *Pseudomonas aeruginosa* showed the liver parenchyma has blood congestions, the hepatic cords were randomly distributions around the central vein. The tissue section showed high aggregations of kupffer cells a rounded the abnormal hepatic cords. The central vein was irregular in shape with very wide lumen compared with the control group (Fig.5). These results were agreement with 22 which reported that many diverse bacterial pathogens share common mechanisms in terms of their abilities to adhere, invade, and cause damage to host cells and tissue, as well as to survive host defenses and establish infection 22.
The histological results of the liver noted many pathological lesions belong to the liver parenchyma. The tissue section showed acute hepatic necrosis in many regions of the liver. The histological results of the liver noted wide cystic dilations in the hepatic parenchyma filled with secretion (Fig.6). The inflammatory cells aggregations were distributed around the cystic dilation. These results were confided with 23. The results appeared most hepatocytes were irregular in shape without a nucleus, and have many vacuoles in their cytoplasm.

CONCLUSION
The histological results showed prominent wide interlobular ducts around the central veins. The tissue sections found acute hepatic degeneration which leads to wide cystic dilation with abnormal hepatic cords. The short hepatic cords have random distribution in different orientations around the portal area. These results were similar which noted the macro examination of visceral organs of the infected group during the 24hr post bacterial injection, noted increased the size of organs especially liver, intestine, and spleen.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES


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