



## RESEARCH ARTICLE

## Influence of Pyocyanin in Murine Lung Tissue

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ARTICLE INFO	ABSTRACT
Received: Feb 22, 2024 Accepted: May 9, 2024	The main objective of the present study was to find out the histopathological effects of pyocyanin pigment at a concentration of 500 µg/ml on lung tissue in male laboratory mice, by extracting and characterizing pyocyanin pigment from <i>P. aeruginosa</i> isolated from environmental samples, and it was injected intraperitoneal male laboratory mice. The Humason method was followed in the preparation of histological sections of lung tissue, and the results of microscopic examination showed the presence of some histopathological changes that increase in viocity with the length of time of the experiment. The results indicate that the cells that suffered necrosis have shown several changes such as the occurrence of pyknosis, enlargement of alveoli cells) hyperplasia (and even necrosis. As well as infiltration of inflammatory cells and the occurrence of congestion and severe bleeding in the lung tissue.
<b>Keywords</b>	
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### INTRODUCTION

Some Bacteria produce pigments that exhibit a number of functions including UV protection, oxidative stress, extreme temperature, and dehydration, and the pigments act as shields that protect bacterial cells from some natural antibacterial compounds produced by other microorganisms (Darshan and Manonmani, 2015; Sajjad, 2020), with regard to the industrial field, there is a trend towards natural dyes because they are safer for human use than synthetic dyes, and more biodegradable, despite the diversity of natural dyes, microbial dyes are preferred because of the ease and speed of their extraction, and the safety of those bacterial pigments such as melanin, pyoferrdin, In addition, the potential medical use of bacterial pigments is strongly associated with their antioxidant, antibacterial, cytotoxic and anti-cancer activities (Numan et al, 2018). Pyocyanin belongs to the family of phenazine with a blue color, active in oxidation and reduction and is one of the secondary metabolites secreted by *Pseudomonas aeruginosa* (*P. aeruginosa*) (Shouman et al., 2023), its molecular formula C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O and its molecular weight is low 210.23 Da (Watson et al. 1986), pyocyanin has the ability to penetrate cell membranes easily and cause the generation of reactive oxygen species, which leads to cellular cycle reduction and a number of cellular changes (Patel et al., 2016). The process of producing pyocyanin is affected by several external factors including temperature, pH, carbon and nitrogen source (Gahlout et al., 2021; Gonçalves and Vasconcelos, 2021).

### MATERIAL AND METHODS

#### Extraction of Pyocyanin

The pyocyanin pigment was extracted from the *P. aeruginosa* isolated from water and soil samples for the districts of Qurna and AL-Medina in Basrah Governorate, by the following steps mentioned by

(El Feghali and Nawas, 2018) with some modifications, and the extraction process was carried out by cultured *P. aeruginosa* on the medium of cetrimide agar and incubating it for five days, followed by a series of steps starting with washing the bacteria and adding chloroform and up to adding HCl and NaOH at a concentration of 0.2 M respectively, and drying the solution to obtain the pigment in powder form.

### **Experimental animals**

The experiment was conducted in the animal house of the Biology Department at the College of Education - Qurna / University of Basrah, where male White laboratory mice used *M. musculus L*, which belongs to the BALB / C strain, weighing approximately 20-24 grams and ages ranging from 10-12 weeks. It was bred with good ventilation conditions and a constant lighting system of 12 hours light / 12 hours of darkness and a temperature of 20-25 °C, placed in plastic cages of standard sizes (45×30×10 cm), and its floor was covered with sawdust and is replaced every two days.

### **Experiment Design**

Male laboratory mice were randomly divided into two groups and each group consists of 15 mice:

**1- Control Group:** It was injected under the intraperitoneal (I.P) at a dose of 0.1 ml of normal saline for 30 days.

**2- Treated Group with pyocyanin:** laboratory mice were injected intraperitoneal (I.P) with a volume of 0.1 ml of pyocyanin pigment at a concentration of 500 µg / ml for 30 days.

The mice were anesthetized by chloroform and dissected according to the periods (4, 7, 14, 21, and 30) days and by 3 repeats in each period of the experiment.

### **Histopathological changes**

The Humason method (1972) was adopted in the preparation of histological sections of the lung of laboratory mice and included a series of multiple steps, starting with the stabilization stage with Bowen's solution and the washing stage with ethyl alcohol at a concentration of 50% to be preserved after that with ethyl alcohol concentration of 70%. The process of water with drawal was carried out by an ascending series of ethyl alcohol at a concentration of (70%, 80%, 90%, 100%, 100%) and was fortified with xylene as a preliminary step for the next stage, which is impregnation and burying the samples with molten paraffin wax at a temperature of 60 ° C. The samples were cut by rotatory microtome and transported to the water bath at a temperature of 42-45 °C and loaded on glass slides coated with Meyer albumin and left until dry to be stained with hematoxylin and eosin dyes.

## **RESULT**

### **Effect of pyocyanin pigment on lung tissue**

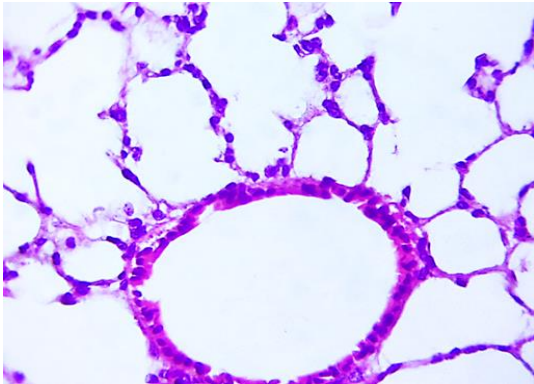
#### **Control group**

The results of histomicroscopic examination of the lungs of mice belonging to the control group showed that they consist of alveoli and are multifaceted pockets with thin walls open from one side in the alveolar sac and separated from each other by the interalveolar septum, and lining the alveoli with two types of epithelial cells type I. They are squamous epithelial cells with a clear nucleus and a thin cytoplasm either type II, they are large cells with chunky nucleoli and sudden cytoplasm, found individually or in small groups, especially in places of the union of septum between alveoli, as in Figure (1).

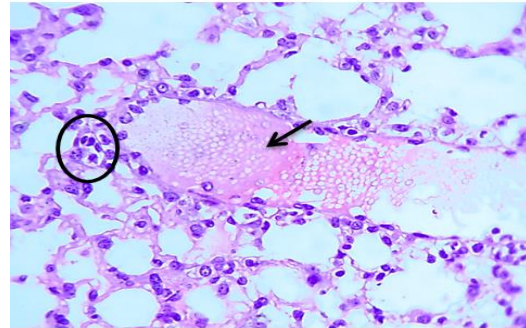
#### **Pyocyanin group**

The results of the microscopic examination of the lung tissue of the group of mice injected with pyocyanin pigment at a concentration of 500 µg / ml showed the presence of some histopathological

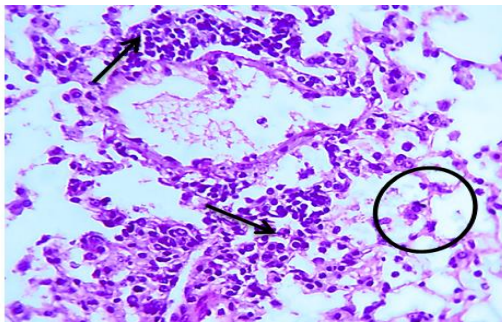
changes, as the results showed after four days of injection the presence of hyperplasia with cytoplasmic degeneration of lung cells and slight congestion within the lung tissue as in Figure (2), and after seven days of injection it was observed necrosis in the alveolar tissue and partial infiltration of inflammatory cells as shown in Figure (3), With alveolar necrosis and hypertrophy and hyperplasia of alveoli cells as in the figure (4), while it was observed that the pyknosis at twenty-one days after the injection as in Figure (5), and the occurrence of severe bleeding and the collection of inflammatory cells and necrosis of the alveoli after thirty days of injection as in Figure (6).



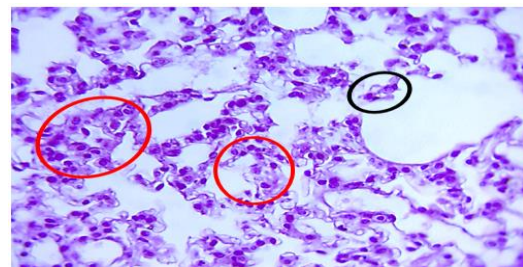
**Figure 1:** Shows the lung tissue and healthy lung alveoli cells in the control group, (400X) (H&E).



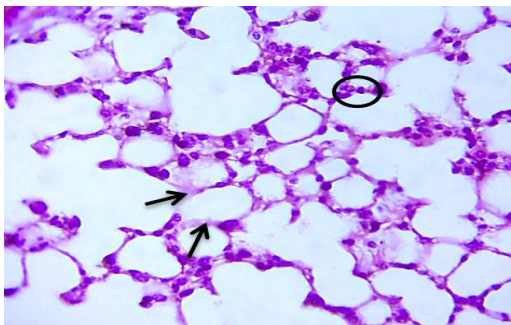
**Figure 2:** Lung tissue 4 days after injection shows simple congestion (arrow) and hyperplasia with cytoplasmic degeneration (circle), (400X) (H&E).



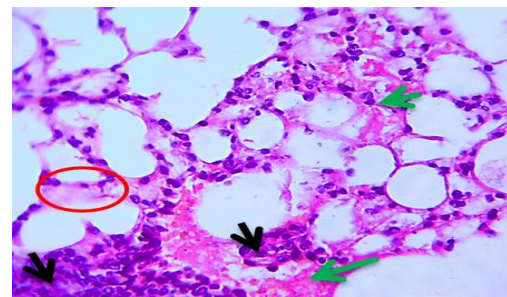
**Figure 3:** Lung tissue 7 days after injection Partial inflammatory cell infiltration (arrows) and alveolar tissue necrosis (circle). (400X) (H&E)



**Figure 4:** Shows lung tissue 14 days after injection and shows alveolar necrosis (black circle) and hypertrophy with hyperplasia alveolar cells (red circle), (400x) (H&E).



**Figure 5:** Shows lung tissue 21 days after injection and shows pyknosis (circle) and necrosis of pulmonary alveoli cells (arrow), (400X) (H&E).



**Figure 6:** Lung tissue 30 days after injection shows acute alveolar bleeding (green arrow), inflammatory cell aggregation and infiltration (black arrow), alveolar necrosis (red circle), (400X) (H&E)

## DISCUSSION

The response of cells exposed to stress or damage varies and that response varies in severity and speed of appearance depending on the type of pathogen, tracteration and period of exposure to it and the type of target cell exposed to that stress or damage, so there is a defect or disorder in the vital activities of those cells that may not be reflected on them sometimes at first and with the continuation of cell stress shows signs of damage to the cells are clear (Cotran *et al.*,1999), the study of lung histological sections in male laboratory mice injected with pyocyanin pigment showed the presence of some histopathological effects, and these changes begin with cytoplasm vicuolization and this condition is considered an adaptation of the pathological condition due to this pigment affecting the tissues (Abdel Hamed, 2004) , as these gaps work to collect harmful substances and prevent them from interfering with the vital activities of those cells (2009, Cheville). The results of the current study showed the occurrence of pyknosis in some areas of lung tissue, and this condition is one of the most prominent phenotypic characteristics of programmed cell death (Karpman et al., 1998). He stated English *et al* (1989) that the cells that suffer necrosis initially show several changes at the level of cytoplasm and nucleus are these changes the Pyknosis and change their shapes and take irregular forms and it turned out that the process of condensation of chromatin is a preliminary step for the process of programmed cell death. Cases of hyperplasia were observed in the cells lining the walls of the pulmonary alveoli, which caused narrowing of the cavities of some alveoli, The prominent thickness of their walls reduces the interchangeability of respiratory gases, and this can be due to the infiltration of the alveoli walls with phagocytes and neutrophils, and this is an adaptation to stimulate the host's defense mechanism as a kind of immune response (Shraideh and Najjar, 2011). The exposure of cells to toxic substances causes inflammatory cases, including congestion of blood vessels in the affected area, causing a lack of processing cells with oxygen and nutrients with the beginning of simple cell necrosis in the area surrounding the inflammatory areas down to comprehensive necrosis, and the changes in it are non-reflexive, and the reason for the occurrence of bleeding, which was observed in a number of areas of lung tissue, can be attributed to inflammation of the lining of blood vessels, as red blood cells are excreted through gaps between endothelic cells to extravascular (Mac sween and whaley, 1992), or it may be due to the effect of toxic substances on cellular communications that connect endothilic cells to blood vessels, as studies have proven that toxic compounds cause obstruction of the process of phosphorylation of proteins, which later leads to a defect in the process of building and polymerizing the components of the cellular skeleton, (Carmichael, 1994), as they are involved in the process of formation and synthesis of cellular communications (Hooser, 2000). Or perhaps the reason for the accumulation of inflammatory cells is due to the presence of residues of necrosis cells and these residues act as attractive substances for defense cells by chemotaxis (Criddle and Ellis., 1994). Due to the low molecular weight of pyocyanin, it can permeate and penetrate cell membranes and thus spread easily, undergo reduction by NADPH and molecular oxygen can then be reduced to superoxide (Zhao *et al.*, 2014), which in turn turns into hydrogen peroxide. Pyocyanin can cause a reaction in eukaryotic organisms at the level of the cell wall and on the mitochondrial respiratory chain resulting in the release of reactive mitochondrial oxygen species, accelerating the aging process and apoptosis (Bonifácio *et al.*, 2020; Manago *et al.*, 2015). Hassan and Fridovich (1980) reported that the toxicity of pyocyanin is due to its ability to undergo the redox cycle in the presence of various reducing agents and molecular oxygen, leading to the accumulation of supertoxic oxide and hydrogen peroxide and thus leading to injury or death of cells.

## CONCLUSION

Based on the results of the current study, the pyocyanin pigment extracted from the environmental isolates of *P. aeruginosa* has some harmful effects on lung tissue at a concentration of 500 µg / ml due to the occurrence of different forms of histopathological changes in male injected mice compared to the control group, including the occurrence of cytoplasmic degeneration with simple congestion and

the severity of these changes increased with the continuation of injections down to the occurrence of severe bleeding and tissue necrosis in other cases.

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