University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 313-250

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/ncmtb379*

Pathological study of pyocyanin that Extracted from *Pseudomonas aeruginosa* that isolated from human with otitis

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Abstract

This study 100 samples were taken from the ear of a person infected with otitis and the positive isolation rate of *Pseudomonas aeruginosa* was 26%, where samples were taken from males (45 cases), the positive was 9 infected samples (20%), and samples were taken from women (55 cases), the result was for 17 infected samples (30.9%). The isolation rate in adults was 34.28% and in children 6.6%, where the isolation was carried out using Cetrimide Media, MacConkey, and Blood Agar and was confirmed by biochemical tests and vitek 2system. Due to the importance of *Pseudomonas aeruginosa*, pyocyanin dye was extracted and It was injected into mice in different concentrations 50µg, 100µg, 150µg and 200µg and the mice were killed after two weeks of injection and the effects of histological pathology show a dense gathering of inflammatory cells in the organs (kidney, liver and lung) in all groups in addition to damage to the renal tubules.and in the lung of groups 150 and 200, severe fibrosis was observed as a result of cystic fibrosis due to pyocyanin.

Keyword :*Otitis, Pseudomonas aeruginosa, Pyocyanin*

I. Introduction

Pseudomonas aeruginosa is a human and animal pathogen that is considered a top 10 superbug (Milivojevic *et al.*, 2018). Because of the emergence of antimicrobial-resistant strains, treatment choices remain severely limited; hence, infection with *P. aeruginosa* remains a potentially fatal risk (Streeter&Katoul, 2016). Nosocomial infections caused by *P. aeruginosa* include meningitis, bloodstream infections, infections of the urinary system, respiratory tract, skin infections, and otitis media (Pachori *et al.*,2019) Otitis media is an inflammatory illness of the middle ear's mucosal covering. Recurrent Otitis media may result in irreversible hearing loss due to injury to the ossicles, facial nerve, and cochlea. It can be either acute or chronic (Tahira*et al.*,2009). *P. aeruginosa* has the potential to manufacture a particularly specific Pyocyanin pigment (PCN) in addition to other pigments, and most studies show that roughly 90 to 95% of *P. aeruginosa* isolates can produce that pigment (Dahah,2017). Studies have shown the possible significance of PCN in the pathogenesis and virulence of pseudomonal infections, as well as its potential toxic consequences (Lau *etal.*,2004). PCN has been demonstrated to have a wide range of adverse effects on the host, both in vivo and in vitro, including pro-inflammatory and free radical effects that cause cellular damage and death (Lau *etal.*,2004). Since cystic fibrosis (CF) patients have a high prevalence of chronic *P. aeruginosa* colonisation, the bulk of PCN research has so far mainly concentrated on the impact of PCN on human airways. However, a number of studies published lately have shown that exposure to PCN may have wider effects, particularly on the CNS, the cardiovascular system, and the urinary tract (McDermott *et al.*,2012), the CNS and the cardiovascular system(Hempenstall *et al.*, 2014).

Aims of study:

Pathological study of pyocyanin extraction from *Pseudomonas aeruginosa* that isolated from human with otitis.

II. Material and methods

University of Thi-Qar Journal of agricultural research

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Samples

The study was based in Baghdad city, in Al-Yarmouk Teaching Hospital. The study population included both gender with different ages. One hundred ear swap samples collected and The samples were sent to the College of Veterinary Medicine's Zoonotic Unit Laboratory under sterile circumstances for further microbiological examination.

Isolation and identifications

Ear swap samples with transport media under sterile conditions, inoculated on Blood agar, Cetrimide agar, MacConkey agar, and nutrient agar (Ibrahim et al.,2022; Qusay *et al.*, 2016). *P. aeruginosa* isolates were identified microscopically using a light microscope to see Gram staining (Quinn *et al.*, 2011), The Gram-negative Identification Card (GN ID) in the Vitek2 compact system, was utilized to confirm the *P. aeruginosa* (Sarah and Zainab, 2023;Al-Malkey *et al.*, 2017).

The procedure for extracting, purifying, and characterizing the pigment generated by *Pseudomonas* isolates is the same as the one described in (Popy Devnath *etal.*, 2018), with a few minor adjustments.

Experimental study:

1-we tack 20 mice divided to 4 groups each one contains 5 mice.

2- Injection I.P with pyocyanin in different concentration (Al-Ani *et al.*, 2020**)**

a- **Group1 Injection 50 µg\ml.**
b- **Group 2 Injection 100 µg\ml.**
c- **Group 3 Injection 150 µg\ml.**
d- **Group4 Injection 200 µg\ml.**

3- After 2 weeks killed all animals.

4- Histopathological examination for internal organs (liver, lung, and kidney

Result:

from one hundred ear swap samples, twenty-six sample show positive for *pseudomonas aeruginosa* (Table 1).

(Table 1): percentage of positive samples for *pseudomonas aeruginosa* isolated from human with otitis.

No. of samples	No. of Positively identified	Percentage%
100	26	26

The result shows, 26 isolates were isolated from the total human samples, results of total samples of infected male and female were 26%, in male (from 45 case) percentage revealed 20% and in female (55 case) were 30.9% that demonstrated in (table 2) below.

Table 2: showed the Isolates distribution of human samples according to gender.

University of Thi-Qar Journal of agricultural research

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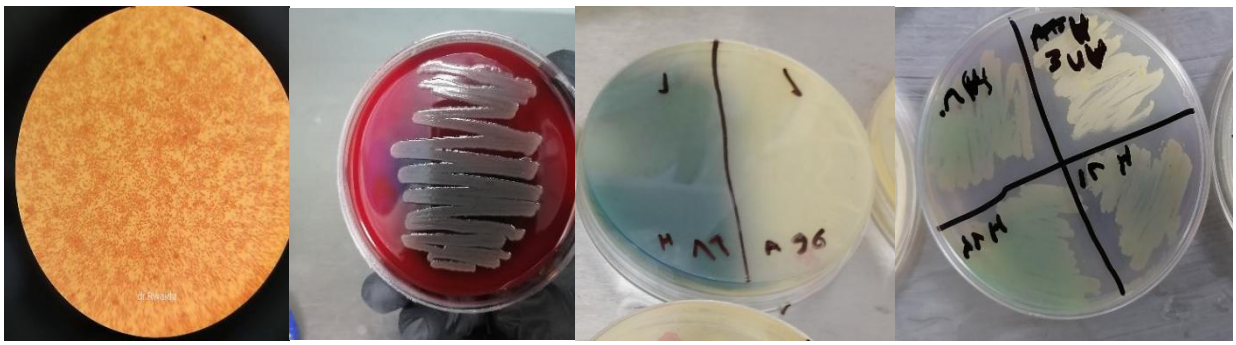
Gender	**Total No.**	**No. of Positively identified**	**Percentage%**
Male	45	9	20
Female	55	17	30.9

And the result showed percentage of positive samples that identified according to stag of age in both genders were 34.28% for adult from 70 case and 6.6% for children (30 case) were demonstrated in table (3) below.

Table 3: showed the Isolates distribution of human samples according to stage of age.

Age stage	Total No.	No. of Positively identified	Percentage%
adults	70	24	34.28
children	30	2	6.6

By use of biochemical testing and microscopic examination, their identification was verified. Also, it was discovered that all of the isolates shared the same traits. The organisms' extensive physiological and metabolic capabilities include catalase activity, sugar fermentation, and growth on a variety of Media (Figure 1) (Figure2).



A B C D

Figure1: A: Gram negative, small rods, arranged as single, pairs or even short chains of *P. aeruginosa* (gram stain X100) B: *P. aeruginosa* on blood agar, the large flat colonies that produced zones of Beta-hemolysis. C: *P. aeruginosa* on nutrient agar show pigment. D: *P. aeruginosa* on cetrimide agar produced pyocyanin pigment

University of Thi-Qar Journal of agricultural research

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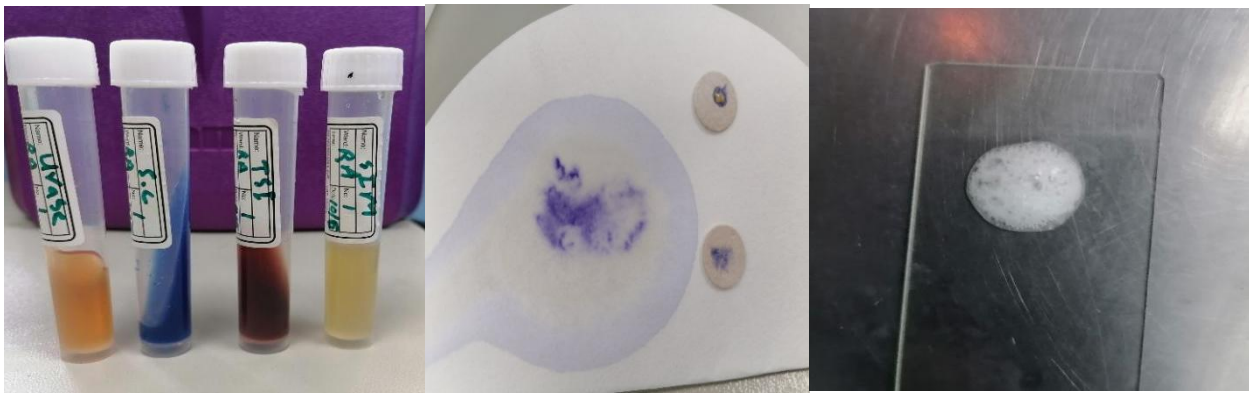

A **B** **C**

Figure2: biochemical testing A: *P. aeruginosa* gives negative urase, H2S and indole, but a positive Citrate and in TSI test (red slant, red butt) reaction: Absence of carbohydrate fermentation results and no acid no gas. B: *P. aeruginosa* positive in oxidase test. C: *P. aeruginosa* positive in catalase test.

Histopathologicl study

1- Group one (injected with pyocyanin 50 µg I.P)

Histopathological section of group 1 after two weeks injected with 50µg of pyocyanin showed aggregation of inflammatory cells in interstitial tissue between renal tubules and glomerulus in kidney as in Fig (3-A), in lung showed aggregation of inflammatory cells around blood vessels and bronchioles presence macrophage and neutrophile (Fig 3-B)liver appeared mild aggregation of inflammatory cells (macrophage) around central veins as in Fig (3-C).

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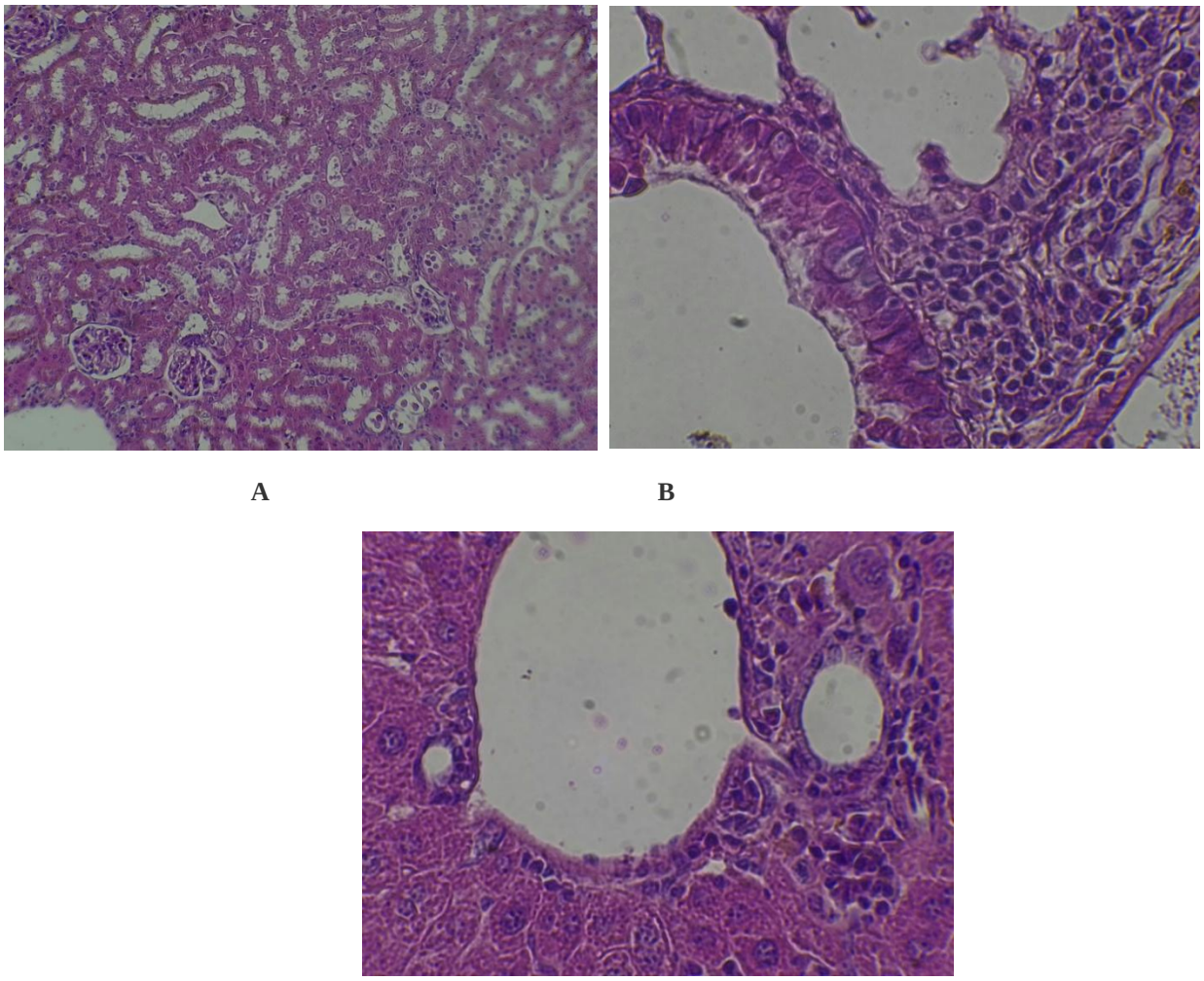

A **B**

C

Fig (3): Histopathological section of group 1 after two weeks injected with 50µg of pyocyanin shows

A-kidney: aggregation of inflammatory cells in interstitial tissue between renal tubules and glomerulus (H&EX100).

B-lung: aggregation of inflammatory cells around blood vessels and bronchioles (presence macrophage and neutrophile) (H&EX400).

C-liver: mild aggregation of inflammatory cells (macrophage) around central veins (H&EX400).

University of Thi-Qar Journal of agricultural research

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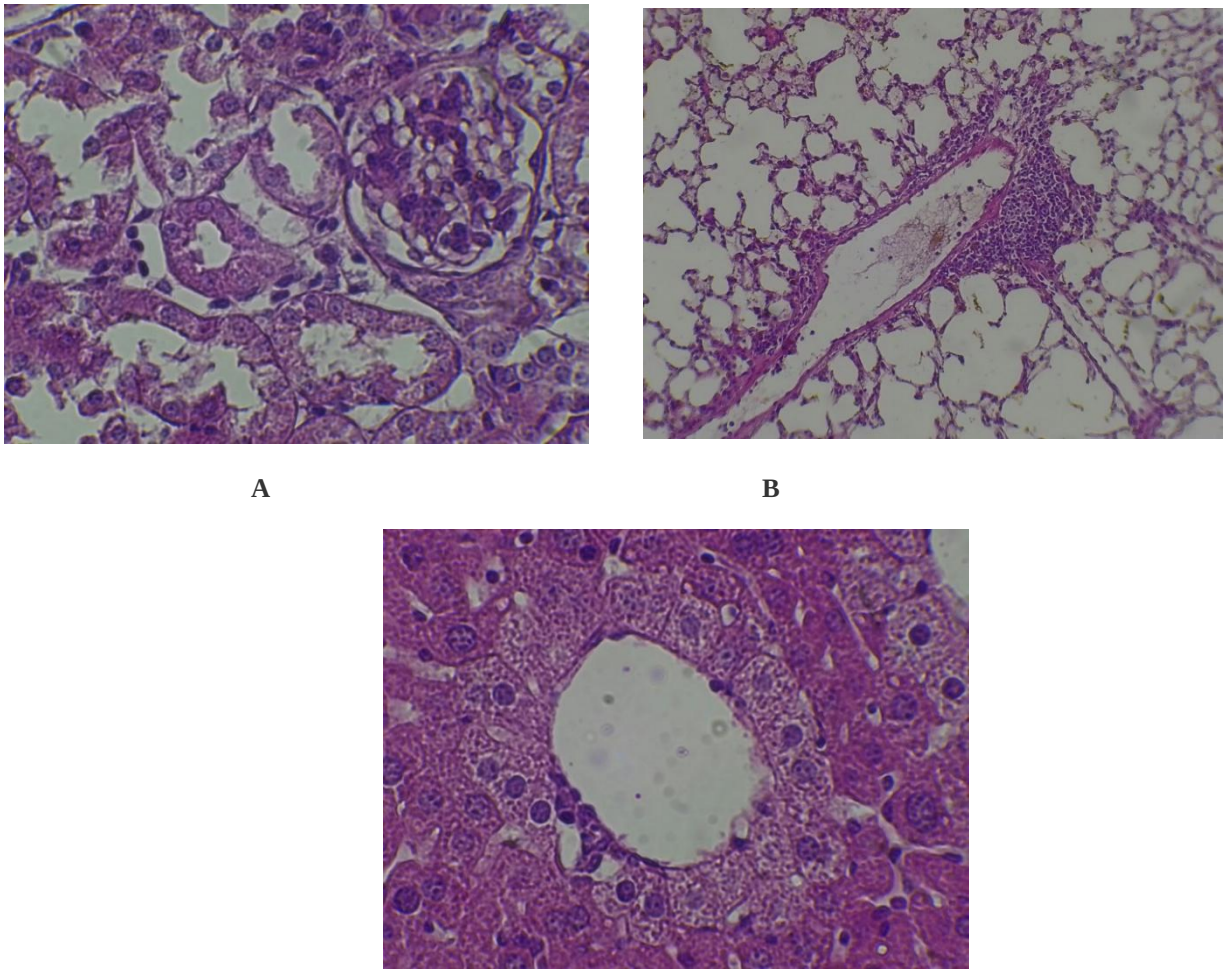
2- Group two (injected with pyocyanin 100 µg I.P)

Histopathological section of group 2 after two weeks injected with 100µg of pyocyanin showed in kidney vacuolation of glomerular tuft, degeneration of some renal tubules with few dissemination of inflammatory cells in renal tissue Fig (4-A), in section of lung showed aggregation of granulomatous lesion around blood vessels and alveolar emphysema in Fig (4-B),and liver appeared apoptosis, cytoplasmic granulation and binucleated hepatocytes around central vein as in Fig (4-C).



A **B**

C

Fig (4): Histopathological section of group 2 after two weeks injected with 100µg of pyocyanin shows

A-Kidney: vacuolation of glomerular tuft, degeneration of some renal tubules with few disseminations of inflammatory cells in renal tissue (H&EX400).

B-lung: aggregation of granulomatous lesion around blood vessels and alveolar emphysema (H&EX100).C-liver: apoptosis, cytoplasmic granulation and binucleated hepatocytes around central vein (H&EX400).

University of Thi-Qar Journal of agricultural research

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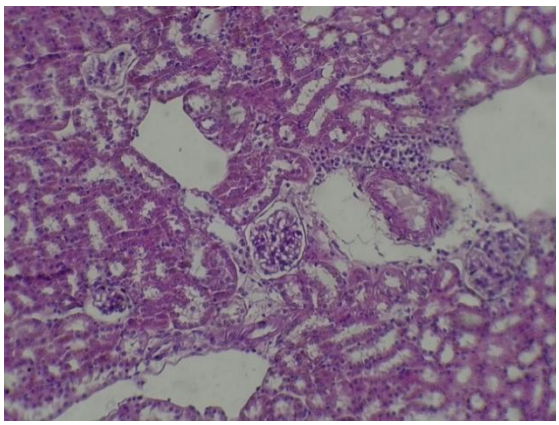
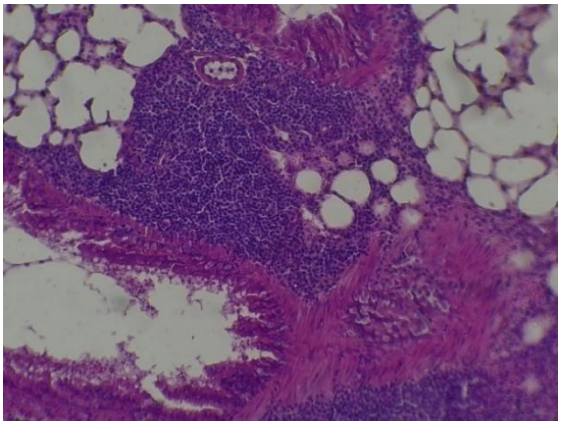
3- Group three (injected with pyocyanin 150 µg I.P)

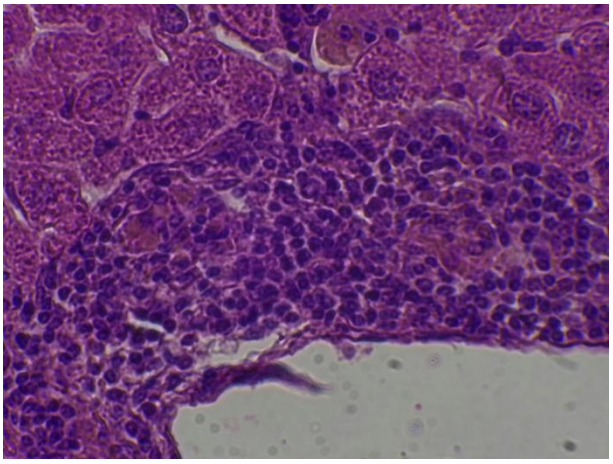
Histopathological section of group 3 after two weeks injected with 150µg of pyocyanin showed in kidney aggregation of macrophage and neutrophiles around blood vessels and glomeruli in Fig(5-A), section of lung showed severe granulomatous lesion around congested blood vessels aggregation in Fig (5-B), liver appeared large lymphocytic aggregation around portal area with apoptosis Fig (5-C).



A



B



C

Fig (5): Histopathological section of kidney of group 3 after two weeks injected with 150µg of pyocyanin shows

A-aggregation of macrophage and neutrophiles around blood vessels and glomeruli X100.

B- severe granulomatous lesion around congested blood vessels aggregation in X100.

C- large lymphocytic aggregation around portal area with apoptosis (H&EX400).

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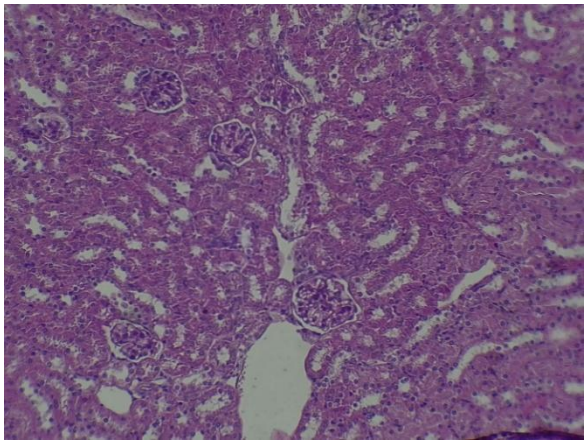
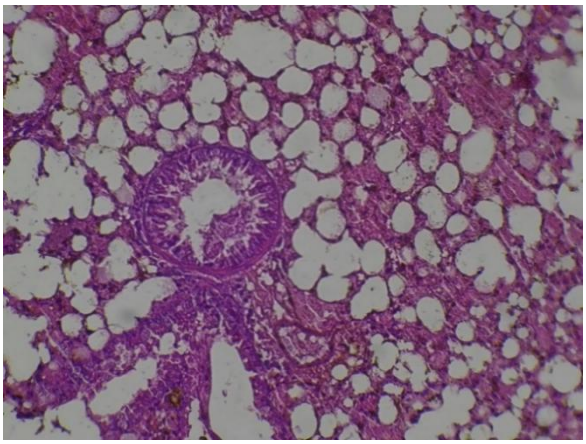
4-Group four (injected with pyocyanin 200 µg I.P)

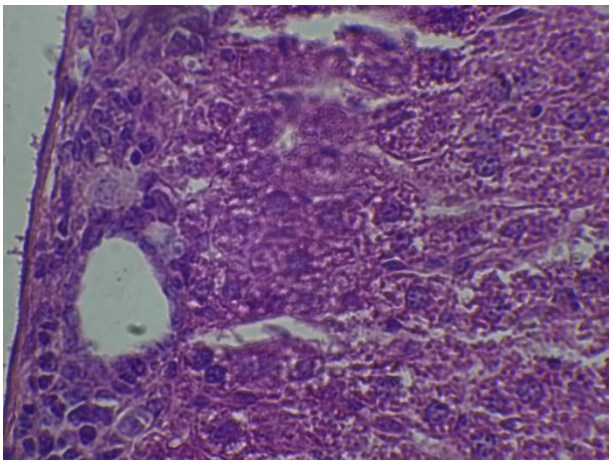
Histopathological section of group 4 after two weeks injected with 200 µg of pyocyanin showed in kidney few degeneration in some renal tubules Fig (6-A), in lung revealed fibrosis of pulmonary parenchyma Fig (6-B), liver appeared apoptosis, granular cytoplasm and binucleated of hepatocytes with some aggregation of macrophage and presence of Kupffer cells Fig (6-C).



A



B



C

Fig (6): Histopathological section of group 5after two weeks injected with 200 µg of pyocyanin shows:

A-kidney: few degenerations in some renal tubules (H&EX100).

B-lung: fibrosis of pulmonary parenchyma (H&EX100).

University of Thi-Qar Journal of agricultural research

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C-liver: apoptosis, granular cytoplasm and binucleated of hepatocytes with some aggregation of macrophage and presence of Kupffer cells (H&EX40).

III. Discussion

this study *P. aeruginosa* is isolated out of ear swap specimens in: blood agar, nutrient agar and Cetrimide Agar as in (Qasim, 2019). And on MacConkey agar to identify non-lactose fermenting activity, and on nutrient agar plates to examine Pseudomonas coloration. The plates were then incubated aerobically at 37°C for 24 hours, same (AL-Shimmary *et al.*, 2018).

Pseudomonas aeruginosa isolates were Gram-negative rods that tested positive for motility, catalase, and oxidase. Yellow green pyoverdine and blue green exopigmentation pyocyanin *P.aeruginosa* growth on cetrimide agar, lactose non fermented on MacConkey agar, and beta hemolysis on blood agar are the same as in (Altaee and Al-Dabbagh, 2022). whereas in (Ahmed, 2015) utilized Cetrimide-Naldixic acid *P. aeruginosa* Chromogenic agar (CNP), yet *P. aeruginosa* delivers the same result in both situations (blue-green pigment of pyocyanin). And the percentage of children infected with *P.aerugenosa* was 6.6%, while the percentage of adults infected with *P.aerugenosa* was 34.28%, because earlier research has indicated that the global pooled incidence of AOM (acute otitis media) is about 11%, with 51% of cases occurring in children under the age of five (Monasta *et al.*, 2012). More than 80% of newborns under the age of three experience at least one episode of AOM; however, only a small minority of children suffer recurrent or chronic OM (COM), with no explanation (Kong, and Coates, 2009) *Streptococcus pneumoniae* and *Haemophilus influenzae* are the most prevalent bacteria recovered from the middle ear of AOM (acute otitis media) patients, with *Moraxella catarrhalis*, *Streptococcus pyogenes*, and *Staphylococcus aureus* being less common. On the other hand, *Pseudomonas aeruginosa* and *S. aureus* are the most commonly detected pathogens in chronic suppurative otitis media (CSOM) (Giebink and Canafax, 1991). And percntage of infected males with *P.aerugenosa* was 20%while percntage of infected women 30.9%, Although there are no sources showing the reason for the high percentage of females over males in the incidence of otitis due to *Pseudomonas aeruginosa*, we can likely do so due to the lack of immunity in general in women due to pregnancy and childbirth, and also in our Islamic society, women wear the hijab, which provides a fertile media for the growth of bacteria where darkness and humidity (Furze *etal.*, 2008).Inflammatory cells were stimulated as there was a dense cluster of inflammatory cells in the organs of groups 50 µg,100 µg,150 µg and 200 µg, where the group of 50 was only a collection of inflammatory cells (neutrophil and macrophage) This indicates the immune response of the body. In the group of 100, in addition to the aggregation of inflammatory cells, there were degenerative changes in the kidney, which indicates an immune response with minor damage to the renal tubules due to byocyanin metabolism and secretion of cytochrome P450 from liver, kidney, and lung; Mature neutrophils move via the sinusoids and are promptly discharged into the circulation after injury or infection, trafficking to areas of infection and/or inflammation (Rankin *et al.*, 2010). Signals from the inflammatory site are required for neutrophil mobilization and recruitment. Tissue-resident cells are activated by a variety of stimuli, such as pathogen-associated molecular patterns and damage-associated molecular patterns, which cause them to release proinflammatory mediators like TNF-a, IFN-g, and IL-1b as well as lipid mediators like LTB4 and neutrophil-specific chemoattractants like CXCL-8 (Zeytun *et al.*, 2010). Neutrophils start "rolling" along the venule walls around the injured area minutes after tissue injury (Butcher, 1991).

Both the cytochromes P450 (CYP) and the UDP-glucuronosyl transferases (UGT) enzyme families are multigenic and play an active role in the metabolism of xenobiotics, which may include medications, pollutants, and hazardous chemicals. CYPs and UGTs are found mostly in the liver, but they can also be found in the lung and the kidney. However, there have been reports of variances in the expression level of these enzymes as well as the types of enzymes that are found in these organs. The fundamental structures, substrate specificities, and responses to inducers of each enzyme are unique to that enzyme (Nelsoin *etal.*, 1993). In groups 150µg and 200µg In addition to the collection of inflammatory cells, there is also severe fibrosis in the lung, which indicates cystic fibrosis, as in the infection with *Pseudomonas aeruginosa* leads to cystic fibrosis in the lung as a result of the virulence factor pyocyanin, this means that pyocyanin alone also led to this disease (cystic fibrosis). that agrees with (Lau *etal.*, 2004).

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IV. Conclusion

The main cause of otitis in humans (chronic) is *Pseudomonas aeruginosa*, and the incidence in adults was higher than in children because they develop acute otitis and also the incidence rate in females is higher than in males due to lack of immunity and women wear the hijab, which provides a fertile media for the growth of bacteria were darkness and humidity. Pyocyanin has large pathological effect on lung, liver and kidney.

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