

Article

Study of the Effect of *Proteus Mirabilis* Bacteria on the Kidney Tissue of Laboratory Mice

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Abstract: This study aimed to investigate the effects of *Proteus mirabilis* on the kidney tissues of laboratory mice. A total of 30 urine samples were collected from female patients with urinary tract infections. The presence of *Proteus mirabilis* was confirmed through bacteriological tests. Mice were orally dosed with *P. mirabilis* over two weeks, and their kidneys were later examined for pathological changes. Histological analysis revealed various stages of degeneration, lymphocyte infiltration, and glomerular damage. The study concluded that *P. mirabilis* induces significant kidney tissue damage, including necrosis, hemorrhage, and congestion, particularly at higher bacterial concentrations.

Keywords: *Proteus mirabilis*, Kidney tissue, Laboratory mice, Urinary tract infection, Opportunistic bacteria

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1. Introduction

Proteus variability bacteria were first discovered by Hauser in 1885, where he isolated them for the first time from feces, sewage and decaying organic matter, and called them *Proteus* because they have the phenomenon of pleomorphism [1]. The genus *Proteus* belongs to the 5 group according to the 1994 Bergy classification [2], which includes the Gram-negative bacillus bacteria and subgroup (1 subgroup). The legal family Enterobacteriaceae and to the tribe known as the tribe Protease [3]. The Protease tribe includes three genera: *Morganella*, *Providencia*, *Proteus* [4] after which the locations of these species changed. In 1950, Henriksen was able to distinguish between the genera *Proteus* and *Providencia* based on biochemical tests, he explained the ability of their production of the enzyme Deaminase, which is not produced by the rest of the intestinal family, and indicated that the genus *Proteus* produces enzymes Lipase and Gelatinase and its inability to produce acid from the fermentation of various sugars such as arabinose, mannose, and mannitol, unlike the genus *Providencia* [5]. *Mirabilis* *Proteus* bacteria are one of the most important species of the genus *Proteus*, which is one of the types of bacteria spread in hospitals and responsible for many of the infections acquired in them. *Mirabilis* *P.* is a microorganism that is opportunistic because of the health problems it causes in

humans [6]. The bacterium mirabilis P. mainly causes urinary tract infection and also causes urinary tract infection in hospitalized patients (and users of urinary catheters). As well as people with abnormal functional and anatomical problems of the urinary tract [7].

Research Objectives

This research includes the following axes:

1. Isolation of Mirabilis P. bacteria and their diagnosis from Samarra City Hospital
2. Study of diseases caused by the bacterium mirabilis.
3. Identification of macroscopic abnormalities and tissue lesions that can be caused by mirabilis p. bacteria in the kidneys of laboratory mice.

Description of the Genus of Volatility

The genus Protease belongs to the intestinal family and to the tribe known as Protease, which includes, in addition to the genus Providencia and Morganella [8], this tribe is distinguished by its ability to remove the amino group of the amino acid phenylalanine producing phenyl pyruvic acid, this test is considered one of the differentiating tests for this tribe from other tribes of the intestinal family [4].

General Characters of Proteus mirabilia

These bacteria are described as short-gram-negative bacilli, with a diameter ranging between (1.0-0.3) μm and a length of (6.0-0.6) μm , this bacteria is characterized by being an optional aerobic that grows under aerobic conditions and moves actively and does not form blackboards [9], also, this bacteria is not constituent of the capsule, and contains (Fimbriae), it also contains Flagellae, negative for oxidase testing, Proskauer vogues, positive for red methyl test, producing hydrogen sulfide gas H_2S when grown on agar iron Klingler medium, as well as can form Pyruvic Phenyl acid when grown on a medium containing Phenylalanine based on the production of the enzyme Phenylalanine deaminase and be positive for the examination of catalysis, some Factors of Virulence Associated with Bacterial Pathogenicity, Bacillus mirabilis p. is widely spread by nature and it can be isolated from humans, animals and water as much as sewage, soil and plants and is considered part of the normal flora of the intestinal tract of humans and animals, Mirabilis p. has a number of pathogenic factors [10]. Among the most important factors of virulence that this bacterium possesses:

1. Urease Enzyme Production

This enzyme is one of the important factors of virulence for this bacterium and it is one of the mineral enzymes (enzymes metalloid) because it contains nickel, whose presence is necessary to form the active site of the enzyme [11].

The enzyme urease is produced from many bacterial alliterations that cause urinary tract infections such as Staphylococcus spp, Ecoli spp, Klebsiella and Proteus spp.

The bacterium mirabilis p. is one of the most important pathogens of the urinary tract producing the enzyme urease, which plays a major role in the colonization process [12]. This bacterium by the action of this enzyme causes infections of the lower urinary tract, but to a lesser extent, this was confirmed by Chlabicz in 2011 in Poland that there were uncomplicated infections by the bacterium mirabilis p. by 3.4%, many studies have shown that the bacterium mirabilis p. has a high ability to produce a large amount of urase unlike other bacteria, and by the action of the urease enzyme, Urea decomposes into CO_2 and NH_3 , leading to a rise in the PH in the urine, which causes the formation of urinary stones [13]. Ammonia is deposited in the form of salts known as Struvite, $\text{MgNH}_4\text{PO}_4 \cdot 5\text{H}_2\text{O}$, and Carbonate apatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{CO}_3$, in the mucous material produced by bacteria and enters

into its cellular structure, which is the substance consisting of polysaccharides and is known as glycocalyx [14].

2. Fimbriae

On the surface of the bacterial cell there are different types of appendages called Fimbriae that have a role in adhering to the surface of the host cell, Adherence is defined as the process of attacking and colonizing the pathogen of the host tissue void, and is the first step in the pathogenesis of bacteria [15], in the process of adhesion, interaction and binding occur between surface molecules on the surface of the pathogen called these molecules called Adhesin or called Adhesive factors [16], or they may be called colonization factors), these adhesives bind to surface receptors complementary to them and located on the surface of the host's cell, the patches are usually lipid proteins or glycoproteins, as for its receptor, it is only sugars, such as: Mannose sugar, that is, the articulators are external structures located on the surface of the cell wall and have an important role in adhesion to the surfaces of cells, this adhesion is an essential step for colonization and infection, especially when urine flows continue [17].

Urinary Tract Infection

Urinary tract infection refers to the presence of microorganisms in the urinary tract although it may be difficult to distinguish between pollution and colonization or infection, Urinary tract infection is one of the common infections in society and affects all age groups, as well as urinary tract infection is common in society and affects all age groups, in addition to the injury of both sexes, males and females, the infection with I.T.U. varies according to age and sex and is more frequent in females than in males at different ages, except for early childhood (less than three months). Where the incidence rate in males is more than in females, and this percentage is (3-5%) in females and (1%) in males, the study reached by Khalaf and Kazim (2012), indicated that the bacterium mirabilis P. is responsible for (11.85%) of urinary tract infections, and it was also shown that the rate of isolation of this bacterium was higher in women than in men, infection can occur in any part of the urinary tract: Kidney, Ureter, Bladder, and Urethra and is classified according to the location of the injury into kidney pelvic infections.

Kidney

Bean-like organ located on both sides of the spine on the back wall of the abdomen and the length of the kidney in an adult human is about 11-10 cm, 5-4 cm wide and 3-2 cm thick, the kidney is surrounded from the outside by a layer of fatty connective tissue, under which there is a layer of Tissue Connective Fibrous on all sides except the Hilus area, from which the blood vessels, lymph, nerves and lymphatic vessels enter and the ureter exits, the kidneys are divided into two main areas, Cortex and are surrounding the site and have a reddish-brown color, and a central area of the site represents Medulla, the blood that reaches the cortex is about 95% - 90% of the blood that enters the kidneys, the cortex contains the corpuscles Renal, the convoluted tubules, the near part of the tubules Straight, the tubules collecting, and ducts collecting, while the pulp contains the remote part of the tubules straight, and ducts collecting, these parts form the nephron, which represents the unit of structure and function in the kidney, which passes from the cortex to the core [18]. The nephron consists of two main parts, Corpuscle Renal and tubeless Renal.

The kidney has a key role in the internal balance of the body, as it regulates the acid-base balance and controls the volume of extracellular fluids and blood pressure, as the kidney performs these functions independently and in coordination with other organs, especially the endocrine system, and the kidney performs many functions, including:

1. Production of resonance hormone when the amount of fluid in the body is low or blood pressure is low, which in turn stimulates the hormone aldosterone, which stimulates the kidneys to retain fluids and sodium ions and reduce urine excretion.
2. Production of vitamin D, which consists of fat.
3. Production of erythrocyte hormone when the level of oxygen is low, which raises the rate of synthesis of red blood cells in the bone marrow, it also contributes to the protection of the free from destruction during their stay in the body.
4. Regulating blood pressure, which is the most important kidney function, and is done by the Renin- Angiotensin System when blood volume or pressure decreases, or the level of sodium in the blood decreases, or when the potassium level rises, Kidney cells secrete a resonance hormone that transforms during multiple processes and in different organs of the body, in the end, it gives the hormone angiotensin, which causes narrowing of blood vessels, which in turn raises blood pressure and stimulates the secretion of aldosterone hormone from the adrenal glands.
5. Production of prostaglandins, which are hormone-like substances made from fat and contribute to stimulating the manufacture of resonance hormone [19, 20].

2. Methods

Sterilization Methods

All ready-made and synthetic implant media and solutions used that are not affected by heat were sterilized by the Autoclave device at a temperature of 121 °C under pressure of 1 atmosphere for 15 minutes, as for the glassware, they have been sterilized in the electric oven at a temperature of 168 degrees Celsius for an hour and a half, heat-affected materials and solutions were sterilized by Milipore Filters [21].

Specimen Collection

The study included the collection of 30 urine samples from patients with urinary tract infection or suspected urinary tract infection, including samples taken from urine, as it was ensured during the collection of samples that the first drops of urine are neglected and the middle amount of it is taken and kept in sterile special collection tubes, the samples were then transferred to the laboratory for transplantation and diagnosis, it was planted on Petri plates containing the middle of the MacConkey agar and the middle of the blood in the planning method, the dishes were incubated at a temperature of 37 °C for 24-18 hours for the purpose of diagnosing bacteria growing on the media.

Isolation and Diagnosis of Isolated Bacteria

Isolates developed on blood and maconki media were diagnosed based on the following bases: culture characteristics and microscopic characteristics.

Biochemical Tests

The following biochemical tests were performed: Ureas test and Citrate utilization test.

Animals Preparation

The animals were obtained from the animal house of the pharmaceutical laboratory in Samarra SDI, it was placed in a room that controlled almost its environmental conditions, the room was air-conditioned and at a temperature of about 25 degrees Celsius,

each three mice were placed in metal cages with one side sliding (slide), the dimensions of each cage were 25 cm in length and 22 cm in width and 18 cm in height and the floor of the cages was covered with sawdust, the animals were given a special diet, the contents of which were feed and mixing at a fixed rate to ensure the provision of all nutritional requirements for the animals, as shown in the table below:

Table 1. Animals preparation

| S. | Percentage | Material |
|----|------------|----------------|
| .1 | 34% | wheat |
| .2 | 20% | Barley |
| .3 | 25% | Yellow corn |
| .4 | 10% | Milk powder |
| .5 | 10% | Animal protein |
| .6 | 1% | Table salt |

The water was provided continuously and through a plastic bottle with a capacity of 500 mL.

Doses Determination

Two doses of man colonies of mirabilis P. were selected, the first was concentrated at a concentration of 0.2, three mice were dosed, the second dose was diluted at 0.1, and three mice were also dosed daily in the morning.

Experimental Design

Eight mice were orally dosed with the stuck containing merabilis P., the doses were as shown below:

1. The first group (control) dosed with distilled water.
2. The second group dosed three females of mice stuck merabilis p. At a concentration of 0.2 and for two weeks daily.
3. The third group in which three females of mice were dosed with merabilis p. at a concentration of 0.1 for two weeks daily.

Anaesthetization and Dissection

The mice under the experiment were drugged with chloroform after two weeks of dosing.

Fixation

The organs to be studied (healthy and affected) were directly fixed with formalin stabilizer for 24 hours and prepared according to the Baker formula [22].

Histological Sections Preparation

The microscopic histological sections were prepared as mentioned in [23] according to the following steps:

1- Washing, 2- Dehydration, 3- Clearing, 4- Infiltration, 5- Embedding, 6- Trimming and sectioning.

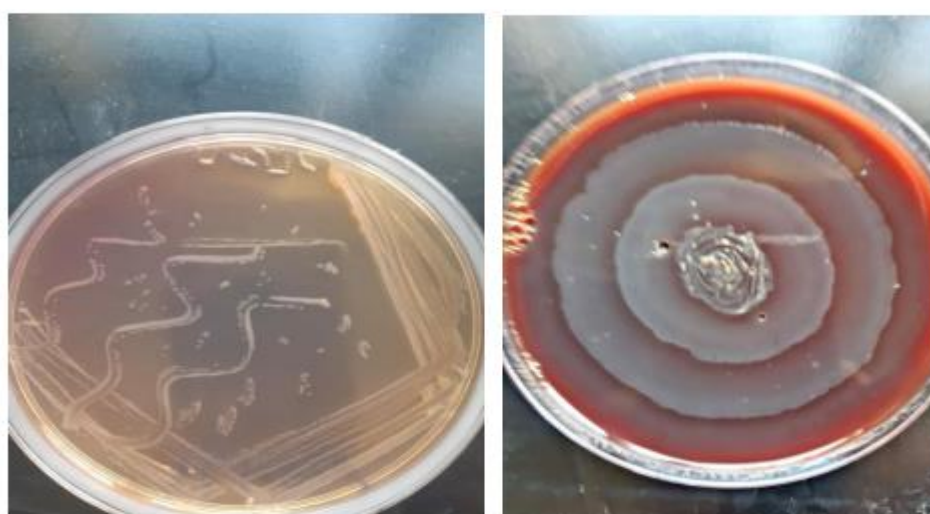
The Mounting

After placing the sections on the glass slide, cover with d. p. x. for the purpose of anchoring, because it is faster to dry, then cover the sections with a glass lid slid cover after placing a small drop of d. p. x, the slices were then left on a thermal surface of 40 °C for the purpose of accelerating drying and then kept in their own boxes.

3. Results and Discussion

Isolation and Diagnosis of Mirabilis Proteus Bacteria

Cultured traits: The results of bacterial isolation showed that most of the isolates that were isolated were for the type of mirabilis Proteus, which is attributed to the great role in urinary tract infection, colonies growing on the middle of the blood acres were characterized by the formation of diffuse growth and membrane growth, which was used to investigate the ability of these bacteria to break down red blood cells and on MacConkey agar was characterized by being small, circular in shape, with a pale brown color, unable to ferment the lactose sugar contained in the composition of MacConkey agar, which contains a neutral red index, therefore, it consumes peptone, which is a source of nitrogen, producing metabolites that raise the basal of the medium, which leads to the transformation of the index color to pale brown.



Planting bacteria on a blood agarwood plate

Agar plate after the growth of cultured bacteria

Figure 1. Isolation and diagnosis of Mirabilis Proteus Bacteria

Biochemical Tests

Urease Test

The results of the biochemical tests were observed that these bacteria showed a positive result for the urease test, thus, it was revealed that these bacteria have the ability to produce the enzyme urease, so the color turns from yellow to red.

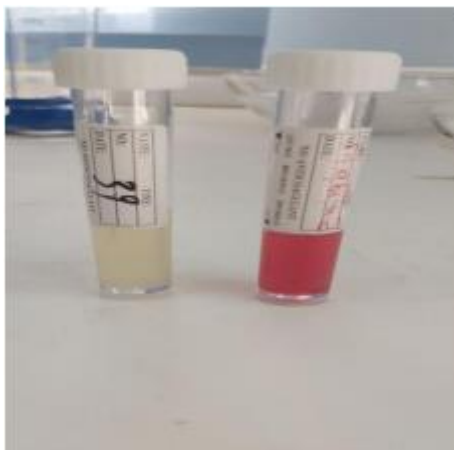


Figure 2. Urease test detection method

Citrate Test

Noted that the result of the Citrate test was positive, so the color changed from green to blue, and this indicates that the result is positive.



Figure 3. Sample before Citrate test



Figure 4. Sample after Citrate test

Phenotypic Changes

Behavioral and Weight Changes

All experimental animals were subjected to direct and continuous observation until the day of the autopsy and the organs were taken, the control group showed normal behavior throughout the duration of the experiment in terms of activity and water and food consumption, the behavioral changes of the treated groups were in the groups treated with the second dose (P.M 2) closer to normal in terms of behavior and nutrition, however, these behavioral changes began to appear in the animals of the groups treated with the third doses (P.M 3) and caused signs of general lethargy, which was represented by little fatigue and tendency to sleep and lack of movement and isolation in one of the corners of the breeding cage with non-resistance (which is present in the normal state) when trying to approach or hold it.

Histological Changes on the Kidney

Group Control

The kidneys of the control group Glomerulus, Proximal convolute tubule and Distal convoluted tubule.

Hypo Dose Second Dose

The clips taken for the kidney tissue after being treated with Mirabilis bacteria were clarified. It is noted that the majority of cuboidal cells lining urinary tubules are normal, but there is a section of tubule cells that have gone through states of cytoplasmic necrosis and nuclear decomposition, as for the glomeruli, there has been a slight contraction and infiltration of lymphocytes and hemolysis, and there are a few areas of the kidneys where congestion occurred, histological examination showed that the boundaries of tubule cells are blurred with each other inside the tubule, and that the cavities of the tubules are normal, there are a few tubules in which cell necrosis has occurred and the cell membrane has ruptured from the upper side facing the cavity so that the cytoplasmic substance spreads in the lumen of the tubule.

Third Dose Hyper Dose

Atrophy occurs in the glomerulus more than the previous group and severe bleeding occurs inside the glomerulus, and the lack of atrophy of the regularity of their cells and the spread of bleeding and congestion in the general tissue of the kidney and the occurrence of decomposition of red blood cells and the large size of the cells of the urinary tubules leading to blockage of part of the lumen of the tubules, most cells are in different stages of decomposition, and the presence of lymphocyte infiltrate, it was noted that the glomeruli are torn in most areas and congestion appears in most areas with irregular Bowman's capsule, and the presence of large infiltration of non-inflammatory cells in the glomerular area and in the areas near it, and the occurrence of blockage of the majority of urinary tubules and the emergence of cases of cytoplasmic necrosis, and the decomposition of the nuclei of the tubules in addition to the increase in cases of degeneration (for the empty at the area of attachment to the basement membrane) it is noted that the plasma membrane in the damaged tubules is thick and visible, while the cells that are attached to the membrane have had necrosis and decomposition.

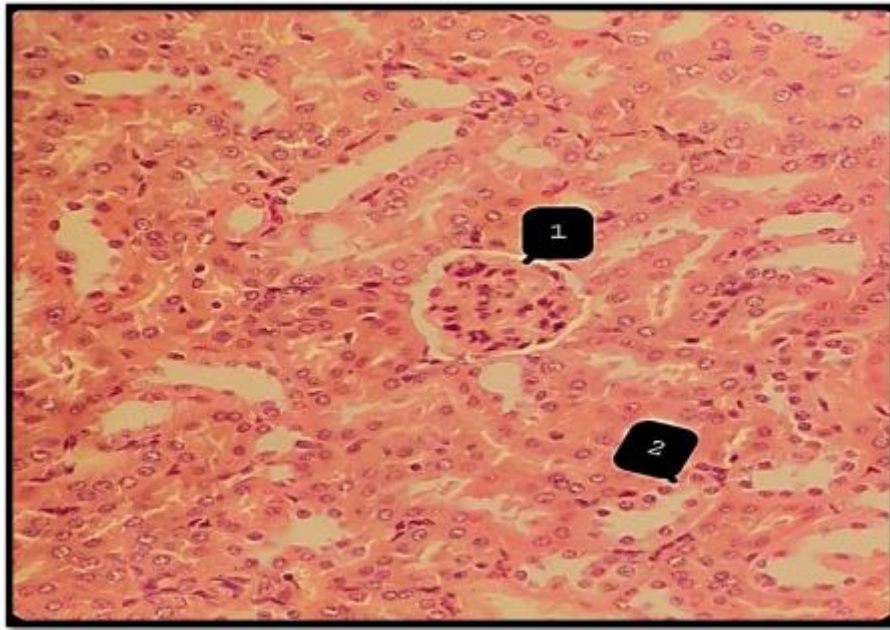


Figure 5. White Mouse kidney control group, showing (1) Glomerular (2) UT renal tubules (dye 400X H&E)



Figure 6. Female kidney dose group II group showing the occurrence of (1) blockage of most of the cavities of the renal tubules, (2) hematological congestion (dye 400X H&E)

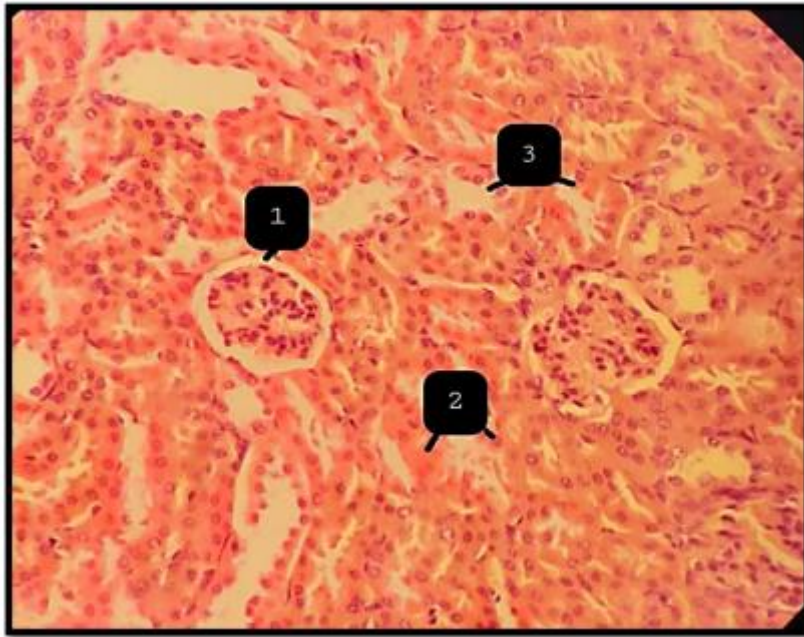


Figure 7. Female kidney mice the second dose group shows (1) glomerular G (2) Hemolysis He (3) Narrowing of the lumen of the renal tubule (dye 400X H&E)

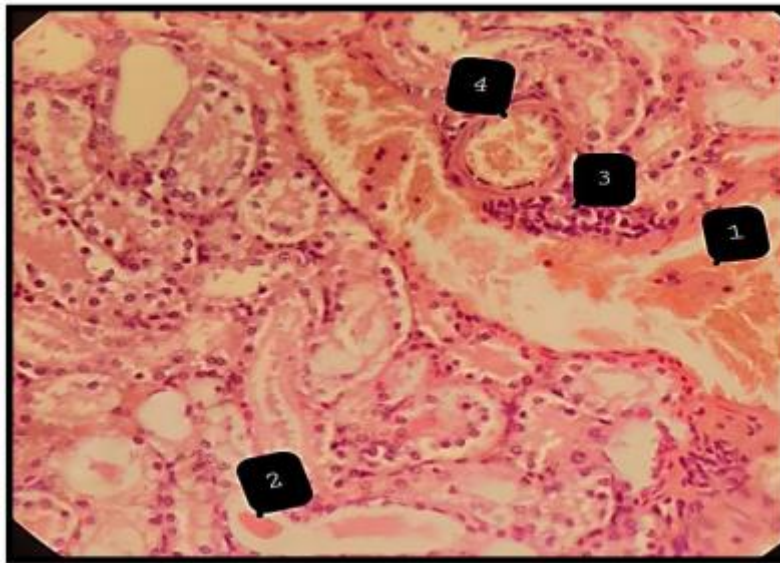


Figure 8. Female kidney group third dose shows the occurrence of (1) CON congestion (2) renal tubule lumen L (3) lymphocyte infiltration IL (4) renal tubule congestion con (400X H&E)

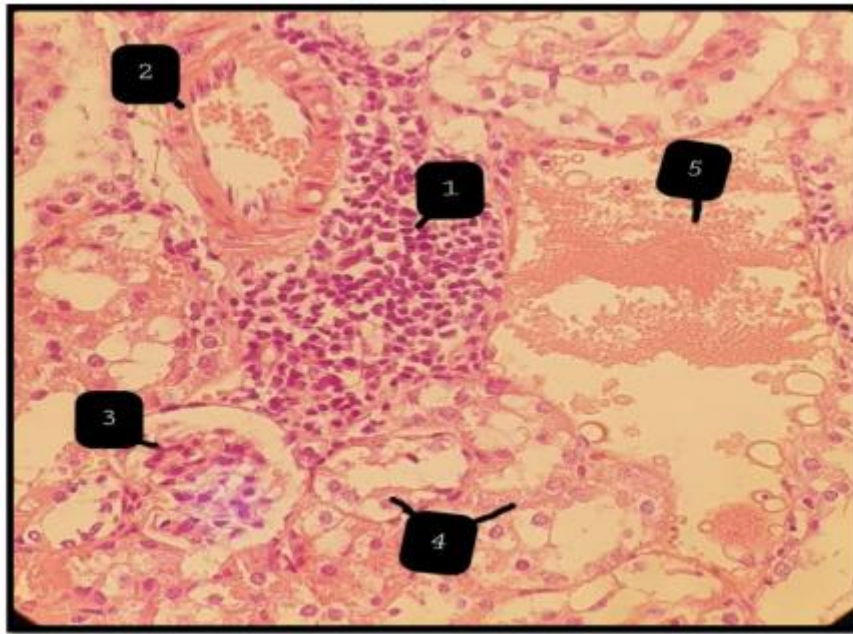


Figure 9. Female kidney group third dose shows the occurrence of (1) lymphocyte infiltration LI (2) glomerular hemorrhage He (3) contraction of the Glomerular Tuft (4) rupture of the renal tubules (5) bloody congestion Con (400X H&E)

Urinary Tract Infection

Urinary tract infection is a condition that arises due to injury to one or more organs of the urinary system, when a type of microorganism can cross a strong natural line of defense, despite the strength of this line of defense, urinary tract infection is common and can occur at any age, there are about 95% of cases caused by the entry of bacteria from the opening of the urethra and spread to the bladder, and in a few cases the bacteria can enter the kidney through the blood [23].

There are many types of bacteria and *E. coli* is an important cause of urinary tract infection, as well as complications caused by proteus bacteria associated with urinary tract infection [24, 25], the ability of the gracious negative bacteria to produce toxins in the lower part of the urinary tract is not fully known [26], however, they may have a number of pathogens that contribute to host patient development [27]. Urinary tract infection is one of the most common diseases in females and most cases of infection are caused by acquired bacteria, treatment of these conditions often begins before laboratory results are available [28]. Urinary tract infection rates increase in young females, as the presence of bloody urination as a symptom of primary infection is a strong indicator.

The results of the current study showed in the kidneys of mice treated with the appearance of tissue lesions represented by the presence of acute cellular swelling with the appearance of different stages of necrosis in the epithelial cells lining the tubules and glomeruli, as well as the presence of blood congestion in addition to the inflammatory manifestations of infiltration of lymphocytes and mononuclear near blood vessels, the severity of these lesions increased at the higher dose of lower doses, the swelling of the cells and the narrowing of the lumen of the tubule may be due to the fact that the toxicity of the virulence factors of the bacteria *P. mirabilis* has led to the cells being enlarged and with a granular cytoplasm and the swelling is so large that it leads to the convergence of the cells to each other and the closure of the lumen of the tubules, the results of our study showed that the toxic effect of bacteria on the urinary tubules and leads to necrosis and then the death of tubule cells because they do not get enough oxygen because their metabolic effectiveness depends on the oxygen supplied by blood vessels, any damage to the blood vessels leads to poor blood flow, and thus a lack of oxygen supply to the cells,

this is consistent with the above, the presence of a large number of large macrophages in addition to the presence of clear necrosis and fibrosis in the kidney tissue in addition to blood congestion, as for the infiltration of mononuclear inflammatory cells, which appeared in this study, it is due to the fact that these cells move towards the higher concentration of some toxic substances, which appears in the form of inflammation.

P. mirabilis, a gram-negative bacterium in the form of oblong rods known for its mass mobility and urease activity, they often cause urinary tract infections associated with catheters (CAUTI), which are often multimicrobial, these infections may be accompanied by urine stones, and the development of bladder or kidney stones due to alkalization of urine from urea-induced hydrolysis, the bacteria are attached to the epithelial surfaces and catheters by different fimbriae milkweeds.

Histological changes observed in the kidneys indicate that endotoxin has led to acute tubule necrosis through changes in the cytoplasm and cell nucleus, the pathological change here begins with the simplest changes, causing a change in the physical position of the cell protein in more specialized cells of the renal tubules [29]. The bacterial toxin usually causes a breakdown of the epithelial wall of tubules and fine cellular details such as mitochondria, especially in the lower twisted tubules of these toxins, which alter the level of ions in the blood, as well as oxygen, which results in a lack of oxygen to the cells and thus the occurrence of local thrombotic necrosis in the tissue, this in turn stimulates through the production of tumor necrosis factors to stimulate the secretion of special chemical mediators to attract inflammatory cells represented by lymphatic and phagocytic and thus be the cause of inflammation in the kidney (Nephritis). This is indicated by this study through pathological changes in the presence of severe bleeding, which indicates the presence of vascular and cellular changes in the tissue through increased permeability [30].

4. Conclusion

1. Behavioral changes were observed for groups of female mice treated with the drug, which are represented by general inactivity and who smelled a little fatigue and the tendency to sleep and lack of movement and isolation in one of the corners of the cage, in addition to the decrease in the level of food intake, the changes have increased with increasing dose concentrations.
2. Kidney changes included contraction of the glomerular lemma and congestion in the overall kidney tissue and increased histological lesions in the third dose.
3. The isolates of *Mirabilis Proteus* bacteria possess a number of virulence factors that enhance their ability to cause infection, it possesses the enzyme urease, hemolysin, bio membrane formation, flagella and milkweed.

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