Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 1, March 2024

Histopathological Changes of *Serratia Marcescens* In Mice

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Received: 4 -11-2023 **Accepted: 16-1-2024** **Published: 1-3-2024**

Abstract:

A prominent opportunistic pathogen that affects a range of hosts, including invertebrates, plants, and mammals, has been identified as the cause of Serratia marcescens, a gram-negative bacillus belonging to the Enterobacteriaceae family. Numerous clinical conditions are associated with it, such as meningitis, pneumonia, keratitis, urinary tract infections, and wound infections; Given that its red colonies are easily identifiable, Serratia marcescens is utilized as a biological marker. The current research sought to investigate the alterations in mice immunized against *Serratia marcescens* infection with (WCSA-S) of *S. marcescens* and (KWCA-S) of *S. marcescens*. Twelve male albino mice were split up randomly into three groups (4 mice for each group) and their histopathological changes examination was performed. The first group was vaccinated with WCSAg-S (500 µg/ml) vaccine subcutaneously (S/C). The third group (the negative control) was given PBS (1 ml) subcutaneously, while the second group received KWCA-S (9 x 10^8cfu/ml) vaccinations (pH 7.2). After 14 days, the male albino mice were given booster doses of the same antigens.. after 28 days of immunized groups with infective dose (1x10^6 cfu/ml) of *Serratia marcescens* in order to determine the histopathological changes in the internal organs (liver, spleen, kidneys, lungs, and intestine) that showed severe histopathological changes in the positive control groups compared with other immunized groups.

Keywords: *Serratia marcescens*, WCSAg-S, Histopathological changes

How to cite this article :

Roa'a Nafie Ahmed ,Ikram Abbas Aboud.Al-Samarraae (2024). "Histopathological Changes Of Serratia Marcescens In Mice." Diyala Journal for Veterinary Sciences **2**(1): 12-27.

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P-ISSN: 2410-8863
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Introduction:

Serratia marcescens (SM) is a Gram-negative pathogen that is an individual from the Yersiniaceae family and genus Serratia. It was first identified in 1819 by Bartolomeo Bizio, an Italian pharmacist. It can lead to a range of infections in people and animals, comprising nosocomial and opportunistic infections (1*). S. marcescens*, once thought to be non-pathogenic, is now recognized to cause infections of the bloodstream, lungs, urinary tract, and eyes (2). Many characteristics of *S. marcescens* pathogenicity and virulence have been established, such as adherence, lipopolysaccharide (LPS), hydrophobicity, and extracellular products. Among these traits is the ability of *S. marcescens* to produce the pigment prodigiosin, which can lead to infection.; many additional characteristics of *S. marcescens* virulence and pathogenicity have been established; by generating lipase, gelatinase, and deoxyribonuclease (DNase), *S. marcescens* typically demonstrates resistance to antibiotics. Additionally, it produces a pore-forming hemolysin called ShIA, which has the ability to induce release of inflammatory mediators and cell cytotoxicity (3). Several stages of the infection process, including colonization and the ability to overcome host defensive mechanisms, appear to be influenced by O-antigen alterations (4).

Colistin, nitrofurantoin, macrolide, tetracycline, cephalosporin, and penicillin are just a few of the many antibiotics to which S. marcescens is resistant; numerous clinical isolates of the bacterium exhibit multiple forms of antimicrobial resistance to these drugs (5). Prodigiosin is a pigment that certain S. marcescens strains are able to produce. Prodigiosin's chemical makeup has been revealed; it was initially employed as a marker to track bacterial activity and transmission because it can activate T-cells and antibodies. (6). Toll-like receptors (TLRs) are a key component of pattern recognition receptors, which are part of the host recognition pathways for both Gram-positive and Gram-negative bacteria. TLRs are specific but also share similar signaling pathways (7). Due to the importance of *Serratia spp*. in the domestic animals as a cause of diarrhea, this research was conducted to determine the protective response through the histopathological changes after challenge with *Serratia spp*.

Materials and Methods:

1- Bacterial Identification:

In Baghdad, Provence, infected fecal sheep samples were used to isolate Serratia marcescens. The samples were then plated onto MacConkey's and Nutrient agar and incubated for 24 to 48 hours at 37 °C. making use of the standard biochemical and morphological tests.

2- Antigen preparation:

The (WCSA-S) and (KWCA-S) of *S.marcescens* were prepared according to (8).

3- Protein concentration detection:

Using the biuret method, the protein concentration of *Serratia marcescens* was determined according to (9)

4- Laboratory animals:

At the start of the experiments, the male albino mice weighed 20 ± 0.5 grams and

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free access to food and water in excess. There were three groups of mice.

5- Immunization of mice:

Twelve male albino mice were split at random to three groups (4 mice for each), as follows: The 1st group was vaccinated with WCSAg-S (500 µg/ml) S/C. The 2nd group was vaccinated with KWCAg-S ($9x10^8$) S/C. The 3rd group was given PBS (1 ml) subcutaneously (the negative control) (pH 7.2). The male albino mice received booster doses of the same antigens after 14 days. The infective dose ($1x10^6$cfu/ml) of *Serratia marcescens* was given after 28 days of immunized groups in order to determine the internal organ histological alterations (liver, spleen, kidneys, lungs, and intestine).

6- Histopathological examination:

Sections measuring 5.6 microns were cut, and haematoxylin and eosin (H&E) stain was applied. 10% neutral formalin was used to fix samples taken from the liver, spleen, kidneys, lungs, intestine, and heart. After that, the soft tissues were cleaned, dehydrated in different amounts of alcohol, cleared in xylol, and embedded in paraffin. according to (10).

Results:

The fecal samples cultured on enrichment, selective and differential media, the biochemical evaluation for *Serratia marcescens* showed that and the positive sample produces red pigment on nutrient agar represent prodigiosin pigment that produced by *Serratia* spp.

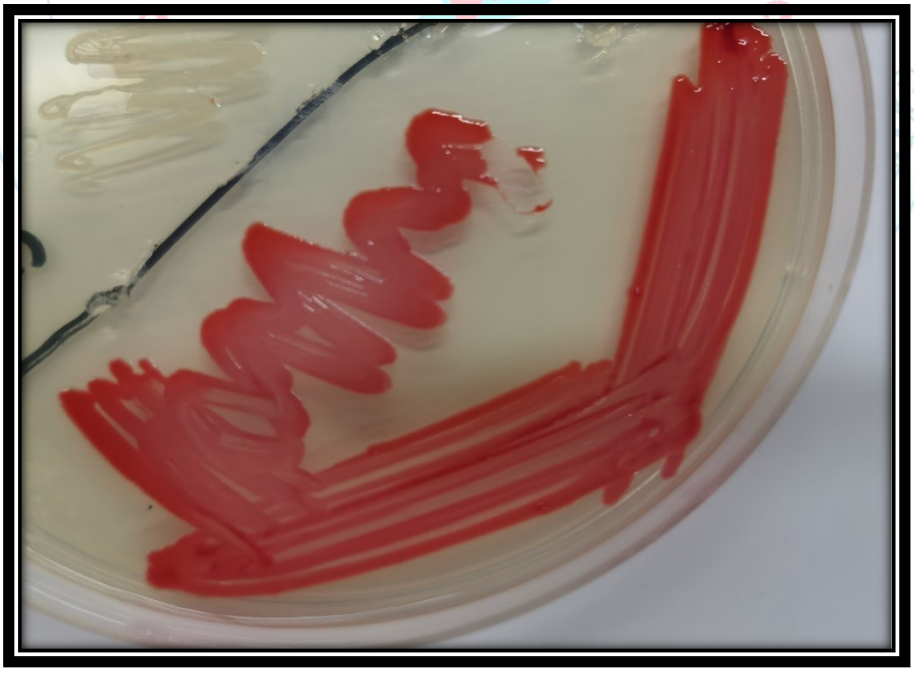

Figure (1): Macroscopic appearance of *S.marcescens* with red pigment on nutrient

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Histopathological changes:

Histopathological examination after 7-days post challenge showed that all examined groups were affected with variable histopathological changes.

The results of histopathological examination of mice infected with infectious dose ($1x10^6$ cfu/ ml) of *S. marcescens* showed varying degrees of histopathological changes from mild to severe. Generally, the lung of mice of the 1st group that immunized with WCSAg-S (500 µg/ml, S/C) showed granuloma consisting from mononuclear cell aggregation in the interstitial tissue with increase thickness of the interalveolar septa as a result of the infiltration of mononuclear cells; the section in the spleen showed hyperplasia of white pulp with amyloid deposition in red pulp; the liver showed Aggregation of mononuclear cells surrounding vascular walls in portal area; the intestine showed no clear lesions while the section of the kidney showed moderate mononuclear cells infiltration in the wall of the glomeruli with mild acute cellular degeneration.

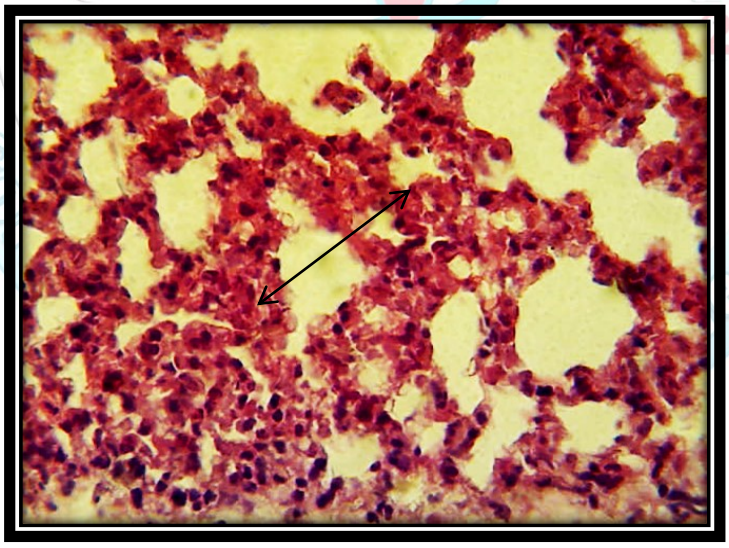

Figure (1): Histopathological section of lung (1st group) after 7 days post challenged showed granuloma consisting from accumulation of mononuclear cells in the interstitial tissue and thickening of the interalveolar septa as a result of the infiltration of these cells ( ) (H & E stain 40X)

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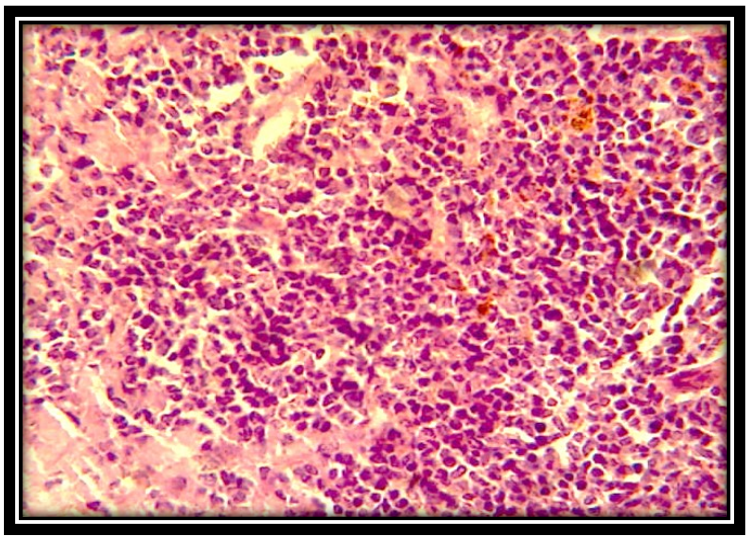

Figure (2): Histopathological section of spleen (1st group) after 7 days post challenged showed hyperplasia of white pulp with amyloid deposition in red pulp

(⟷ (H & E stain 40X). ⟷

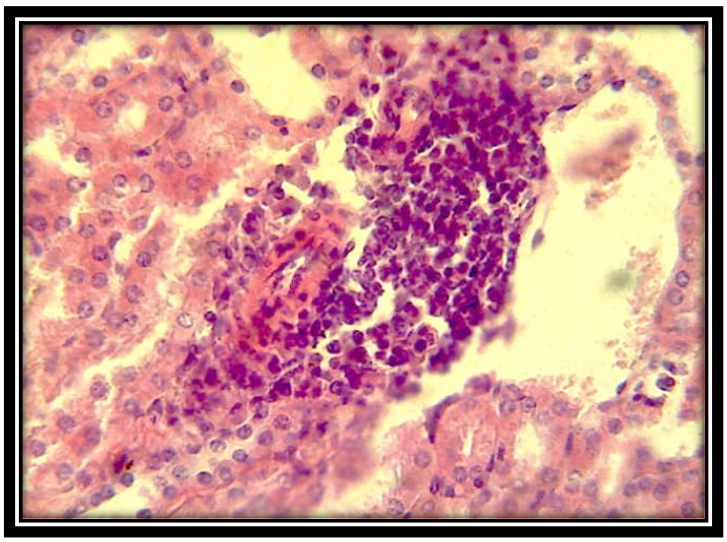

Figure (3): Histopathological section of liver (1st group) after 7 days post challenged showed mononuclear cells aggregation around blood vessels in portal area ( ) (H & E stain 40X).

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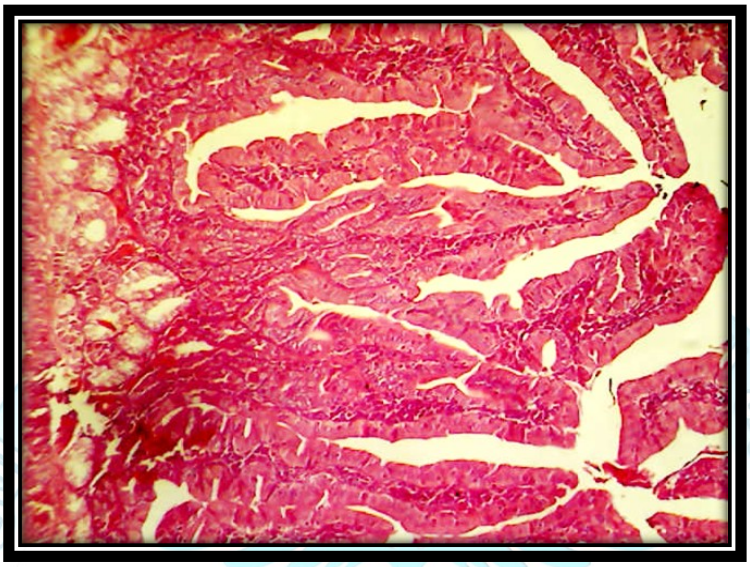

Figure (4): Histopathological section of intestine (1st group) after 7 days post challenged showed no clear lesions (H & E stain 40X).

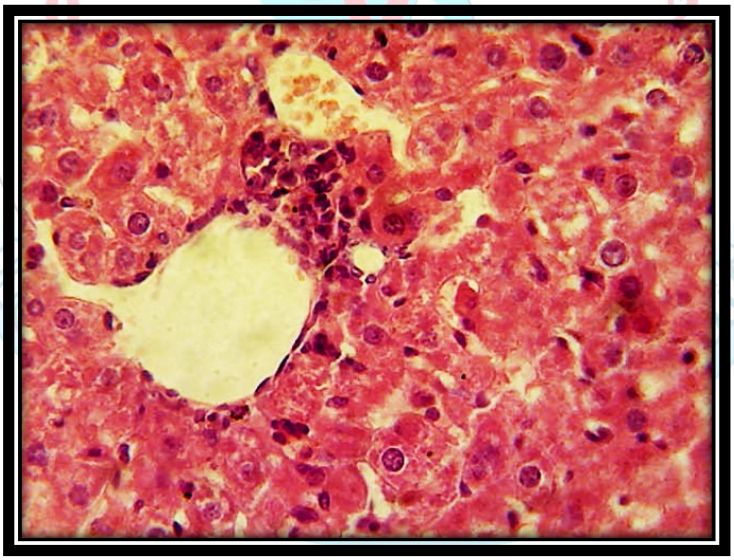

Figure (5): Histopathological section of liver (1st group) after 7 days post challenged showed mononuclear cells aggregation in the wall of central vein and clogged blood vessel ( ) (H & E stain 40X).

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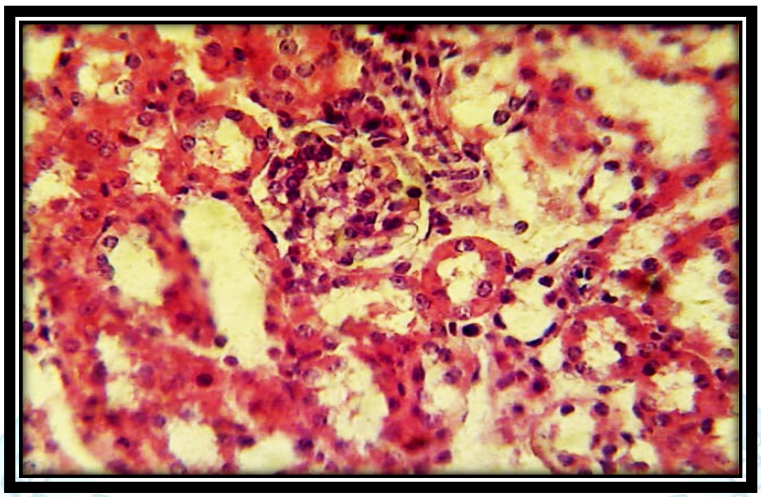

Figure (6): Histopathological section of kidney (1st group) after 7 days post challenged showed moderate infiltration of mononuclear cells in the glomerular wall with mild acute cellular degeneration (H & E stain 40X).

The results of 2nd group of mice that immunized with KWCA-S (9 x 10^8 CFU/mL) immunization showed, the section of the kidney defined by severe mononuclear cells infiltration in the glomerular wall with mild acute cellular degeneration, the heart showed moderate mononuclear cells infiltration in the pericardium, the main microscopic examination of the lung revealed hyperplasia of lymphoid tissues around blood vessels and bronchioles, the intestine showed no clear lesions while the liver showed granulomatous lesions.

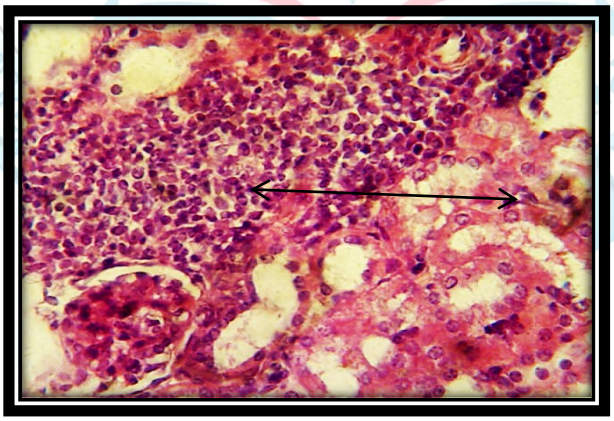

Figure (7): Histopathological section of kidney (2nd group) after 7 days post challenged showed severe e mononuclear cells infiltration in the glomerular wall with mild acute cellular degeneration.

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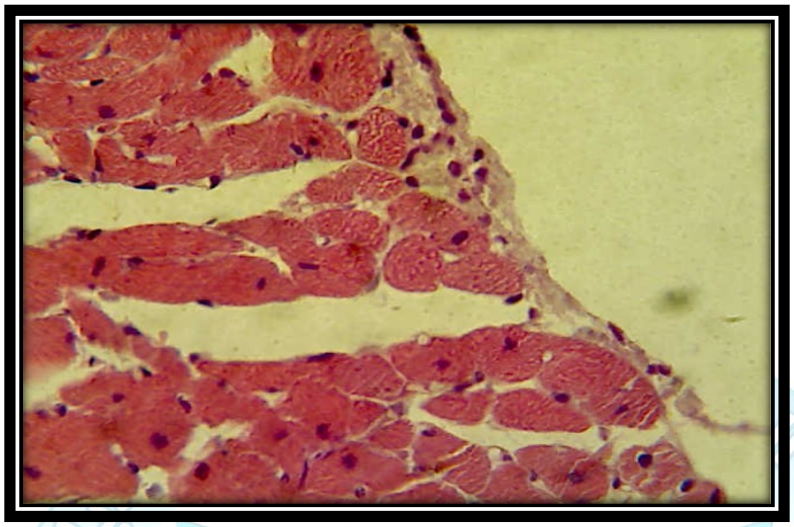

Figure (8): Histopathological section of heart (2nd group) after 7 days post challenged showed shows moderate mononuclear cells infiltration in the pericardium (H & E stain 40X).

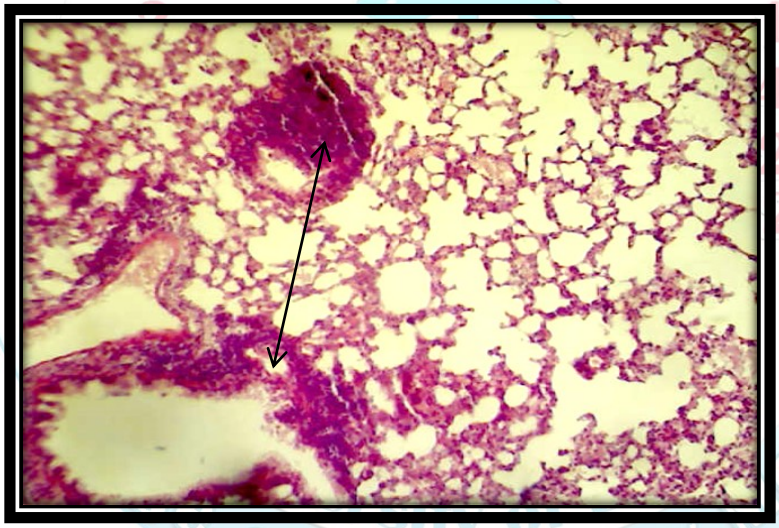

Figure (9): Histopathological section of lung(2nd group) after 7 days post challenged showed hyperplasia of lymphoid tissues around blood vessels and bronchioles ( ) (H & E stain 40X).

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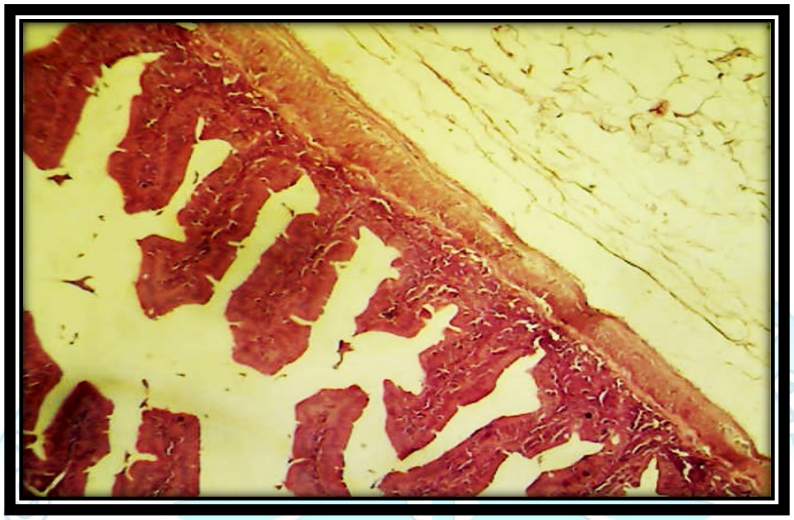

Figure (10): Histopathological section of intestine (2nd group) after 7 days post challenged revealed no obvious lesions (H & E stain 40X).

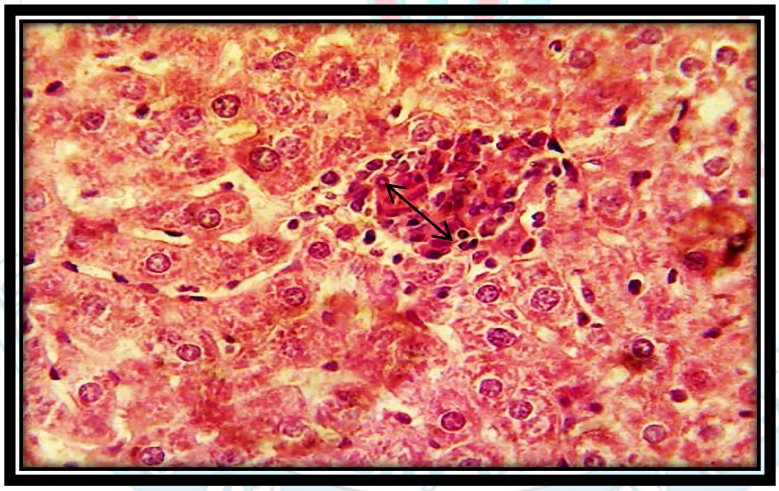

Figure (11): Histopathological section of liver (2nd group) after 7 days post challenged showed granulomatous lesions (H & E stain 40X).

The results of 3rd group of mice that injected with PBS (1 mL) subcutaneously revealed by the section of the spleen showed apoptosis of lymphocytes in white pulp left multiple spaces filled with cellular debris; the section of the lung characterized by deposition of networks of fibrin with cells that cause inflammation, especially neutrophils in alveolar spaces; the section of the liver showed necrosis of hepatocytes with inflammatory cells particularly neutrophils and mononuclear cells in and

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sinusoids while in another section of liver revealed by inflammatory cells infiltration in the liver parenchyma; the kidney showed hyper cellularity of glomerular tufts with fibrosis of Bowman's capsule; the intestine showed inflammatory cells in clogged blood vessels while the section of heart showed inflammatory cells infiltration and edema between cardiac muscle.

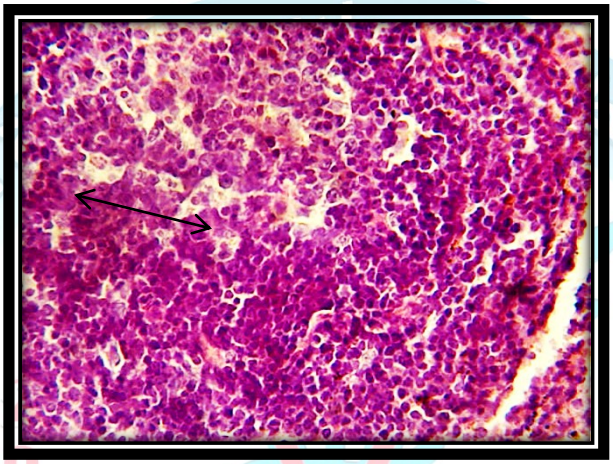

Figure (12): Histopathological section of spleen (3rd group) after 7 days post challenged showed apoptosis of lymphocytes in white pulp left multiple spaces filled with cell debris ( ) (H & E stain 40X).

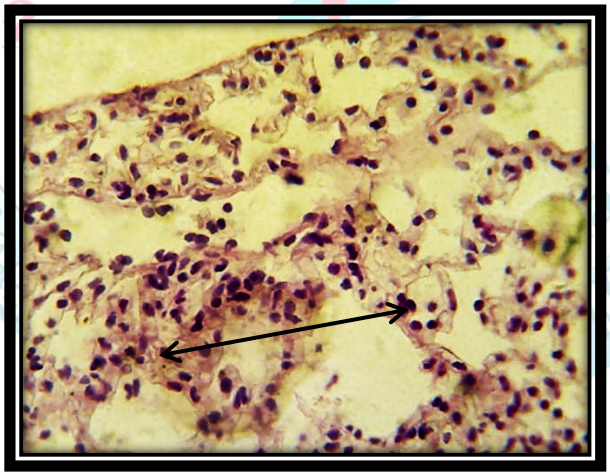

Figure (13): Histopathological section of lung (3rd group) after 7 days post challenged showed fibrin networks deposition with cells that are inflammatory in alveolar spaces ( ) (H & E stain 40X)

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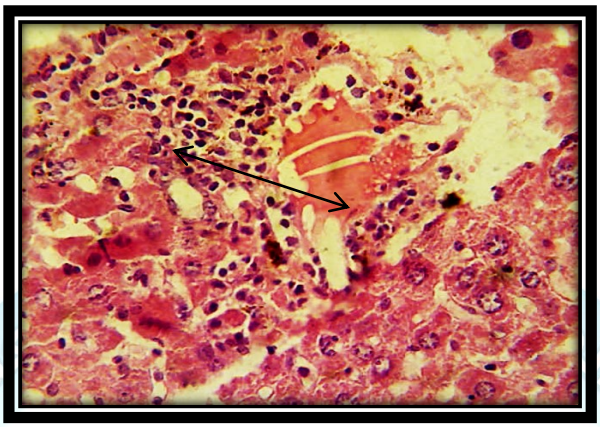

Figure (14): Histopathological section of liver (3rd group) after 7 days post challenged showed necrosis of hepatocytes with inflammatory cells particularly neutrophils and mononuclear cells in and around clogged blood vessels and in sinusoids ( ) (H & E stain 40X).

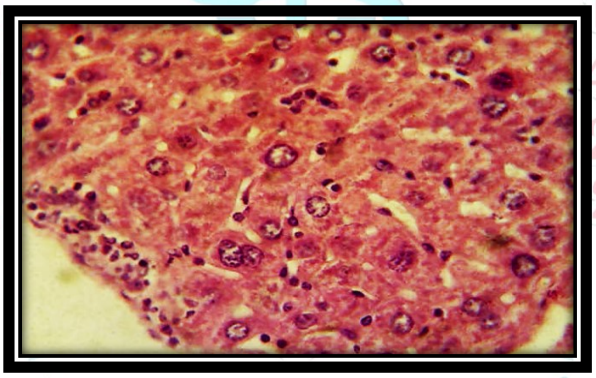

Figure (15): Histopathological section of liver (3rd group) after 7 days post challenged showed inflammatory cells infiltration in the parenchyma of liver (H & E stain 40X).

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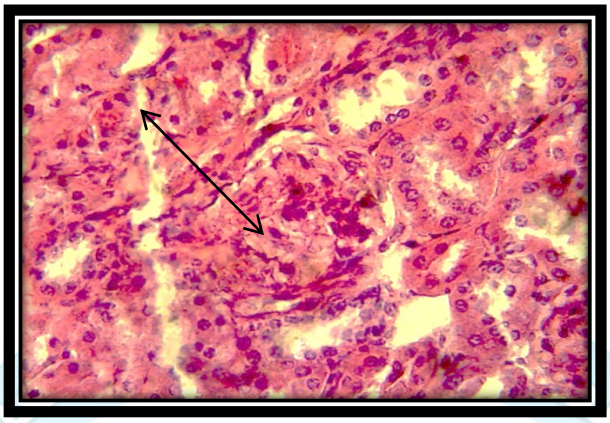

Figure (16): Histopathological section of kidney (3rd group) after 7 days post challenged showed hypercellularity of glomerular tufts with fibrosis of Bowman's capsule (↔) (H & E stain 40X).

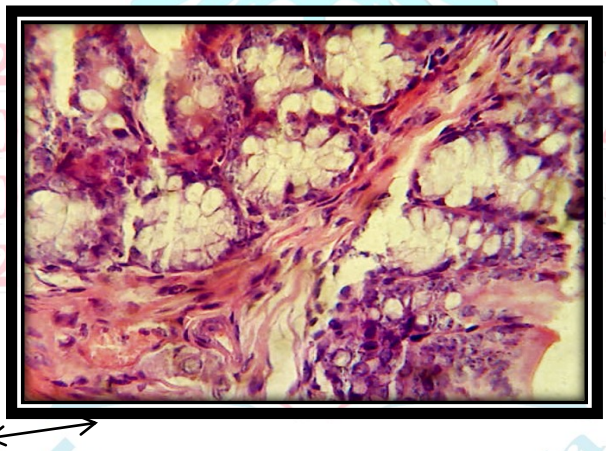

Figure (17): Histopathological section of intestine (3rd group) after 7 days post challenged showed inflammatory cells in congested blood vessels (↔) (H & E stain 40X)

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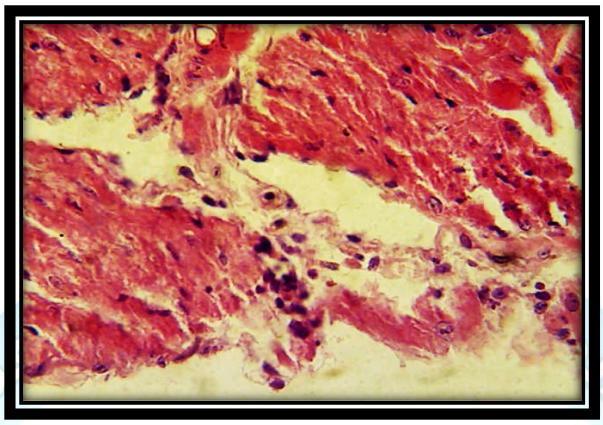

Figure (18): Histopathological section of heart (3rd group) after 7 days post challenged showed inflammatory cells infiltration and edema between cardiac muscle (H & E stain 40X).

Discussion:

The current research's findings suggest that immunizing male mice against *Serratia marcescens* activates their immune systems in comparison to control groups that have severe histopathological lesions.

The research's administered mice underwent histopathological analysis, which showed that the bacteria had a noticeable impact on various tissues with the presence of inflammatory cells like neutrophils, lymphocytes, and macrophages.

S. marcescens is significant opportunistic pathogen like other enterobacteriaceae infect several sites, including urinary tract, respiratory tract and showed acute cellular infiltration in urinary tract and proliferation of lymphocytes in bronchial associated lymphoid tissues; also there is kupffer cellsl proliferation and mononuclear cell aggregation around the liver's central veins additionally, congestion of the blood vessels and infiltration of inflammatory cells in lumen (11).

This research is in agreement with (12) and (13); they found that *Serratia spp*. must be taken into consideration as a possible bacterial pathogen for vertebrates, and *Serratia spp.* infection resulted in histological alterations in the organ tissues of diseased individuals. Also this research was approved by (14) who investigated that the mice as a appropriate model for the proposal and experimentation of products with immunobiology for either passive or active immunization, given that the immunological and histopathological alterations in *E. coli*-infected mice and proposed that EPEC triggers immunological responses and alterations in intestinal histology.; also this research is in agreement with (15) which found that systemic *S. marcescens* infection commonly cause splenomegaly although this increase in splenic cellularity is often due to the recruitment of leukocytes. The type of strain and injected antigens had an impact on the levels of cellular immune response;

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furthermore, the vaccine's ability to protect the animal against a virulent challenge was linked to the strain's ability to establish itself in the liver and spleen (16). CD 4 T cells are representing as the specific cells for *S. marcescens*. Such cells localize to the peyers patches of the small intestine (17). The type of strain and injected antigens had an impact on the levels of cellular immune response; furthermore, the vaccine's ability to protect the animal against a virulent challenge was linked to the strain's ability to e in the liver and spleen (18). The histological sections were similar to (González-Juarbe et al.,2015) who described that the mice forced to aspirate a clinical *S. marcescens* isolate intratracheally experienced pneumonia that was strikingly similar to human pneumonia in terms of both bacterial persistence and histopathological evidence of bronchopneumonia, which included reduced lung function and extensive tissue consolidation; mice with the infection showed a significant pulmonary hemorrhagic response to the infection, which grew worse when they were neutropenic (19, 20).

Conclusions:

Previously, *S. marcescens* was considered less-pathogenic, but now they are considered highly virulent for domestic animals and human; sonicated antigens are potent antigens that induced higher humoral and cellular immune response than killed sonicated antigen however, both antigens induced humoral and cellular immune response alternately.

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Vol. 2, NO. 1, March 2024

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