Evaluation of the Immunogenicity of Salmonella typhimurium Flagellar (H) Antigens and their Effect on the Tissues of White Male Rats

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Abstract

Fifty blood samples were collected from patients suspected of having typhoid fever, and all clinical blood samples were subjected to serological diagnosis of salmonella, where 30 positive samples were obtained with a percentage of 60% and 20 samples negative with a percentage of 40%, and after isolation we noticed the absence of growth for four samples with a percentage of 13.3%, while 26 samples with a percentage of 86.6% showed a positive growth, which were carried out by phenotypic, microscopic, biochemical diagnosis and diagnosis using Vitek 2 Compact technique. 11 isolates were obtained with a percentage of 42.3% for S Salmonella typhi, 10 isolates with a percentage of 38.4% for Salmonella typhimurium, and 5 Isolates with a percentage of 19.2% for Salmonella paratyphi. The flagella antigen was extracted for S.typhimurium, and the lethal half-dose (LD50) test result showed that it was 1×10-4 cell/ml. The results of the phagocytic index (PI) for detecting the efficiency of flagella antigens showed an increase in the phagocytic index with the increase in the dilution of flagellated antigen,a high indicator of phagocytosis with an average of 71.205, the 10-4 dilution was the highest indicative with an average of 75.50, at the same time the dilutions showed a high reading of the Arthus index with an average of 2.040 and the delayed hypersensitivity (DTHR) with an average of 2.086, in which the 10-4 dilution was the highest reading with an average of 2.611 and 2.665, respectively. To confirm the high immunogenicity of this antigenic dilution, this dilution was used in the experimental immunization, which showed many secret signs that appeared on the rats, such as diarrhea, lethargy, high temperature and a high antibody index with an average of 160 mg / liter. The tissue slides showed the effect of all the organs studied, and the appearance of infections, infiltrates, ruptures of the cytoplasm of cells, and an increase in the proportion of blood cells and macrophages, which shows the acute immune reaction resulting from immunization with flagella antigen and its toxic effect on some organs.

Keywords. Salmonella typhimurium, Flagellar(H) Antigens, Immunogenicity, histological effects and male rats

1. Introduction

Salmonella typhimurium is a Gram negative bacilli [1], borne by Peritrichous flagella [2], its flagella consists of the protein Flagellin (FliC) [3], which consists of approximately 490 amino acids that differ among its serotypes [4], and gives bacteria the ability to swim in different fluids and fluids [5], and enables bacteria to respond to changes in the external environment through the organized movement of flagella resulting from the rotation of the flagella motor by the chemical information contained in the membrane receptors [6], and is also important for adhesion on the host cells and their invasion [7], flagella may be identified as pathogen associated molecular patterns (PAMPs) that the host uses to identify bacteria and in addition to their function of locomotion [8], flagella of *S. typhimurium* are highly stimulators of the innate and adaptive immune systems, as they activate the receptor Extracellular Toll-like receptor 5 (TLR-5), which leads to an inflammatory response that stimulates the production of cytokines [9]. Flagellar antigen is a protein that contains many immune components and is affected by heat [10], and it is not stable with alcohol, but formaldehyde solution has a good preservation for it with a concentration ranging between 0.04-0.2%, and it can be removed by heating for 30 minutes at 100 °C [11], but still immunostimulating antibodies in many salmonella species [12], and this antigen in many salmonella species is found in two phases, Phase1 and may be present in one or more species of Salmonella bacteria called the specialized phase, while the second phase, many salmonella serotypes share the antigen it is from the same phase and is called the non-specialized phase [13]. In this study, salmonella bacteria were isolated from the blood of patients with typhoid fever, and the flagellar antigen of *S.typhimurium* was prepared and its immunization efficiency was evaluated and its effect on the tissues of white male rats was studied.

2. Materials and Working Methods

2.1. Sample Collection

Fifty samples were collected from patients suspected of having been diagnosed with typhoid fever, sleeping and returning to Shirqat General Hospital during the period from August 20, 2021, to December 1, 2021, and 5-10 ml of venous blood samples were collected using sterile syringes and placed in special tubes until use. Serologically diagnosed using the diagnostic kit (Febrile Antigen Salmonella _PLASMATEC_UK) and the reagents contained *Salmonella Typhi* O, H and Salmonella antigens and were used according to the

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manufacturer's instructions.

2.2. Bacterial Isolation and Diagnosis

Samples were grown by plotting method on MacConkey agar medium, then grown on blood agar medium, XLD medium and SS agar medium, and then incubated for 18-24 hours at 37°C. Bacterial isolates were purified as single colonies were selected from the first culture media and replanted by plotting again on new XLD plates for the purpose of obtaining more pure isolates [14], and the isolates were subjected to microscopic diagnosis according to the description [15], and biochemical tests were performed that included oxidase and catalase tests as mentioned [16], and IMViC tests [17], then the diagnosis was confirmed by Vitek 2Compact System (BioMerieux).

2.3. Preparation of lagellar antigen (h antigen)

The method [18] was used, and it is summarized as follows:-

1- The active colonies were selected after purification on the semi-solid medium of the movement, prepared from the nutrient broth medium plus 0.2% of agar.

2- 1 ml of bacterial yield was added to 500 ml of brain and heart infusion medium, and incubated at 37 °C for 18 hours.

3-An equal volume of phosphate saline containing 0.4% formalin at a concentration of 40% was added to it to fix the flagella on the surface of the cells.

4- On the next day, a sterility test was conducted by planting samples on solid blood medium and Maconkey medium and incubated at 37 °C for 24 hours, to ensure that the cells were killed, as well as to ensure the integrity of the bacterial crop and not contamination, then it was examined with a gram stain.

5- After making sure that no growth appeared in the above culture media, the bacterial cells were precipitated by a cold centrifuge at a speed of 10,000 rpm for 10 minutes, washed with a phosphate saline buffer and re-sterilized.

2.4. Determination of the toxic half-lethal dose of s. Typhimurium in rats

The LD-50% was determined according to the [19] method of my agency:

1-The prepared antigens were diluted decimally using phosphate-buffered saline, and were divided as follows:

A- The flagellated antigen group 5 decimal dilutions are: 10^{-1},10^{-2},10^{-3},10^{-4},10^{-5} and 30 rats were used, with 6 rats for each dilution.

B- The control group that was injected with physiological solution and used 4 rats.

2- Withdrawal of food for 24 hours and each group dosed orally by 1 ml of dilution while the last group dosed 1 ml microliter of physiological solution.

2.5. Antigen efficiency test

The immune response was assessed in rats using the LD-50% antigens. Three rats were used for each dilution and tested as follows:

2.5.1. Phagocytic index

It was carried out according to [20] as the rat was injected in the peritoneum with 0.5 ml of each dilution of antigen dilutions and the injection area was carefully rubbed for 3 minutes, then the rat was left at room temperature for 7 minutes, then injected in the same place with 0.5 physiological saline solution and the injection area was massaged After that, the injected saline solution is withdrawn and transferred to a test tube. A drop of the mixture is taken and placed on a glass slide. The slide is dried with air and stained with Giemsa dye for 15 minutes, then rinse the slide with distilled water, leave at room temperature to dry and examine the slide with a 100X oil lens. The phagocytosis index (PI) is calculated according to the following equation:Phagocytosis index (PI) = (phagocytic cell number) / (total cell number) ×100.

2.5.2. Arthus and hypersensitivity index

The left footpad was injected intradermal with 0.05 mL of antigen dilution, while the right footpad was injected with 0.05 mL of normal saline. Four hours later, the thickness of both pads was measured using a Vernier, the difference being the Arthus reaction index, which was measured in units of millimeters (mm), the thickness is then re-measured after 24 hours, and the difference between the two pads is an indicator of delayed hypersensitivity reaction (DTHR) [21].

2.6. Experimental immunization of rats with a semi-lethal dose of flagellar antigen

Run according to the method prepared by [22] by my agencies:

1- 16 rats were used, and they were divided into two groups, The food was withdrawn for two days before infection, and Amikacin 10 Mg and Azithromycin 15 Mg were dosed using the gastric tube, and based on the results of the sensitivity test, the bacteria showed high resistance to these drugs.

2-Drinking water is naturally boiled and cooled, and these drugs are added to it with the following concentrations: Amikacin 4 Mg/ ML Azithromycin 4 Mg/ ML and it was continued throughout the duration of the experiment, and the two groups of the experiment were divided as follows:

A-group flagellated antigen: This group includes 10 rats, they are immunized orally with 500 µl of (10^{-4}) flagella antigen, and after 14 days they are immunized orally with a booster dose for the first dose and in the same quantity, and after 28 days the challenge dose is given, and left for 3 days before killing and autopsy.

B- group control: This group includes 6 rats, not immunized with any of the antigens and represents the control sample.

3- The animal groups were followed up and monitored during the treatment period by examining or inspecting the changes in each of: the general condition of the animal, the nature of the appetite, the nature of the skin, the nature of the stool, the temperature rate, the pulse rate and the respiratory rate, and comparing that with the indicated natural rates. In [23], a diagnosis of immune reaction was carried out in male rats immunized with *S.typhimurium* antigens using the diagnostic kit (Febrile Antigen Salmonella _PLASMATEC_UK) for the confirmatory diagnosis of the immune reaction that was conducted throughout the days of the experiment.

2.7. Preparation of tissue sections

The histological study was conducted on the following organs (intestines, liver, spleen, kidney, heart and lung) after the rat was anesthetized and killed. The organs were fixed directly in 10% formalin solution for 24 hours, and the tissue pieces were prepared according to what he mentioned [24].

2.8. Statistical analysis

It was conducted according to the description [25] and using SPSS according to the (One Way ANOVA test), with a probability level of $p < 0.05$.

3. Results and Discussion

Fifty blood samples were collected from patients suspected of having typhoid fever in Sharqat General Hospital, who were suspected of having typhoid fever (the clinical diagnosis was based on the presence of some symptoms such as fever, headache, loss of appetite, nausea, vomiting, abdominal pain with diarrhea or constipation for 6-18 days and underwent all clinical blood samples were for the serological diagnosis of salmonella, as 30 positive samples were obtained, with a percentage of 60%, while 20 samples showed a negative test with a percentage of 40%. The positive samples were subjected to the examination for isolation and bacterial diagnosis, which showed the absence of growth for four samples with a percentage of 13.3%, while 26 samples showed with a positive growth rate of 86.6%, the growing samples were divided between 11 isolates and 42.3% of *S.typhi*, 10 isolates and 38.4% of *S.typhimurium*, and 5 isolates and 19.2% of *S.paratyphi*, It is noted in Table 1.

TABLE 1. Isolation and diagnosis

Diagnostic type	Positive samples		Negative samples	
Serological diagnosis	Number	Percentage%	Number	Percentage%
	30	60%	20	40%
Growth diagnosis	Growing samples		Non-growing samples	
	Number	Percentage%	Number	Percentage%
	26	86.6%	4	13.3%
Bacterial diagnosis	Bacteria		Number	Percentage%
	S.typhi		11	42.3%
	S.typhimurium		10	38.4%
	S.paratyphi		5	19.2%

From the above, we find that the percentage of positive samples (86.6%) and negative (13.3%) that our study showed was not compatible with the result obtained by Al-aarajy [26] who obtained a growth rate of 39.7% and a percentage of 60.3% non-growth, as for the percentage of isolates of both *S.typhi* 42.3%, *S.typhimurium* 38.4% and *S.paratyphi* 19.2% in our study, they also did not agree with Al-Badri et al. [27] who obtained 14.4% of his isolates for *S.typhi* and 25% of his isolates for *S.typhimurium*. Our results are close to Al-Saffar [28], which obtained 9% of its isolates of *S.typhimurium*, and our results differed with Al-Naimi et al. [29] who found the highest isolate rate of *S.typhimurium* at a rate of 56.9%, Sharp and Riilly [30] attributed the reason for the increase in the spread of Salmonella spp. on another due to the different environmental conditions as well as the characteristics of the species and its ability to resist the environmental conditions in the environmental area. While Al-aarajy [26] attributed the reason for the difference in the incidence of Salmonella spp. between studies to the natural feeding pattern and the lack of appropriate health conditions. This is also consistent with Jenkins et al. [31]. Boyd et al. [32] suggested the reason for the high proportion of Salmonella spp. in the blood of infected people to the virulence of this bacteria and to provide it with many mechanisms that allow it to cross the intestinal barriers and infect the blood. *S. typhimurium* is one of the causative agents of enteric fever and is the most important cause of typhoid fever in Iraq and elsewhere. The high false-positive rate may be due to reactive antibodies to other bacterial infections such as *S. enteritidis* or other non-intestinal Salmonella infections [33], or the antibodies may be caused by From an old infection, because the Widal test does not distinguish between acute infections and old infections. The incidence of typhoid fever varies during the year in Iraq, which is expected, because typhoid fever in Iraq is a seasonal disease, and the majority of cases occur in the summer months [34].

The study showed that the 10^{-2} dilution caused the death of 50% of the rats experimentally dosed with 1 ml of *S.typhimurium* flagella antigen, while the 10^{-4} dilution did not cause the death of any of the rats, thus the LD50 is the half lethal dose. As can be seen in Table 2.

TABLE 2. shows the number of dead and alive for each dilution of the flagellated antigen.

Dilutions	Deaths	Revivals	Percentage
10-1	3	1	75%
10-2	2	2	50%
10-3	1	3	25%
10-4	0	0	0
10-5	0	0	0

The rats used in this test were sensitive to the LD50 of 10^{-4}, and this result did not agree with what was obtained by Zhao and his group [35] who found that the MLD was 1×10^{-7} cell/ml. The reason for the death of rats may be attributed to Goh et al. [36] that the flagella are highly virulent antigens that have receptors on the surfaces of the cells, which causes an acute immune response that results in the death of mice in the case of high doses, and flagella is a major target and an effective stimulator for the production of antibodies and macrophages upon infection [37]. It was found that *S. typhimurium* flagella cause rapid death of host cells [38]. Goh et al. [36] supported in their study the idea of a vaccine based on flagella, and indicated that *S. typhimurium* flagella could be a promising inhibitor to gain the body the necessary immunity when infected with Salmonella spp., this was confirmed by Zhao et al. [35]. It was found that eradicating the flagella of *S.typhimurium* leads to inhibition of its pathogenicity and limits its ability to invade and use hosts [39]. The dose needed to induce disease depends on the strength or virulence of the bacteria, especially when it carries resistance plasmids that include resistance stomach acidity or antibiotic

resistance, as well as the dose, affects the host's exposure to previous infection or the host's administration of antibiotics [40].

The results of the phagocytosis index (PI) for detecting the efficiency of flagella antigens showed an increase in the phagocytic index with the increase in the dilution of the flagella antigen, it showed a high index of phagocytosis with an average of 71.205, the dilution was 10^{-4} the highest indicator with an average of 75.50, at the same time the dilutions showed a high reading of the Arthus index with an average of 2.040 and DTHR with a mean of 2.086 had the highest reading 10^{-4} dilution with a mean of 2.611 and 2.665, respectively, which confirms the immunogenicity of the flagellated antigen with this dilution, as noted in Table 3.

TABLE 3. Arthus index and hypersensitivity to flagellated antigens

Dilutions	Phagocytic Index	Arthus	DTHR
10-1	67.16	1.350	1.387
10-2	69.50	1.860	1.941
10-3	72.66	2.341	2.354
10-4	75.50	2.611	2.665
Overall average	71.205	2.040	2.086
Negative control	39.00	0.450	0.400
Positive control	67.50	1.680	2.166

Fahey et al. [41] concluded in their study that the capsular antigen of *K. pneumonia* is able to increase the phagocytic index (PI) and increase the presence of pulmonary macrophages in the peritoneum, as well as stimulate the release of TNF-∞, IL-1 and IL-6., Or the Arthus reaction. It mediates the emergence of an immune complex with the antigen to result in the immune complex between the antibody and the antigen in the injection site, which leads to activation of the complement system, which results in inflammatory edema 3-4 hours after the injection [42, 43]. Delayed-type hypersensitivity reaction mediating the cell reaction in which T-lymphocytes play a major role in binding with macrophages occurs 24-48 hours after antigen injection, this time required for T-lymphocytes activation by the macrophage-borne antigen [44, 45].

Experimental immunization was started using a half lethal dose LD50 (500 µl at a concentration of 1 ×10^{-4} cells/ml) in the experimental group of rats, which had some clinical signs indicating the effectiveness of immunization, including poor appetite, diarrhea, lethargy and high temperature, and the serological examination showed a positive result during six days of the first immunization dose and four days after the booster dose, the average antibody measurement was 160 mg / liter, which indicates the effectiveness of the dose of flagellated antigens and the occurrence of an immune reaction in the group of rats dosed, while no signs of disease appeared on the group. Tennant et al. [46] reported that oral administration of vaccines provides protection to mice against a lethal challenge dose of *S. typhimurium*, and this may be attributed to the protection resulting from the cross-reactivity of the cellular immune response that effectively stimulates these oral vaccines [47].

The histological anatomy of the intestine showed that the mucosal layer contained villi lined with simple columnar cells with some goblet cells, and the base plate of the villi contained infiltration of leukocytes with a disintegration of the soft connective tissue in the pulp of the villi, and infiltration was found in leukocytes around the muscle layer of the intestinal wall with the presence of some macrophages, as noted in Fig. 1, and the surfaces of the intestinal villi contained a thickening of the small villi, as well as degeneration of some epithelial cells lining the villi. As for the outer smooth muscle layer, it has extensive erosion of the cytoplasm of muscle fibers and lymphatic nodular infiltration and other cells diffusely, and the muscle layer is surrounded by from the outside with adipose tissue, as can be seen in Fig. 2. This result is close to what Al-Hadithi [48] of goblet cell hyperplasia of rat lung, epithelial cell laceration, filling of the intestinal cavity with mucus and inflammatory cells, and congestion of blood vessels in the base plate.

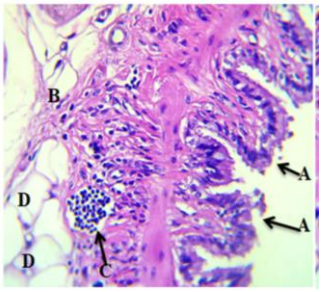

FIGURE 1. Intestinal villi lined with simple columnar cells. (A) Goblet cells (B) infiltration of leukocytes into the villi pulp and primary lamina (C) infiltration of leukocytes around the muscular layer with macrophages (D)CH2 EY 40

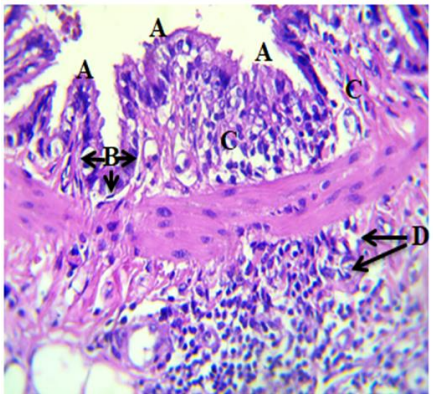

FIGURE 2. Intestinal villi and the degeneration of it's epithelial cells (A) Eruption of the cytoplasm of muscle cells of the intestinal wall (B) Lymphatic nodular spread (C) Adipose tissue (D) CH2 EY 40

The liver parenchyma contained hyper-enlargement of cells that appeared compactly with each other and blood granules between the liver cells appeared in the form of a network of protrusions containing kupffer cells and erythrocytes. The portal area contains the bile duct branch and portal vein branch containing blood clot and dissolved blood, surrounded on the outside by infiltrating leukocytes and macrophages, as seen in Fig. 3. Circumference of the liver contained hyper-enlargement of hepatocytes with coagulation of other cells and thickening of the nuclei of some hepatocytes. Kupffer cells were found in hematopoietic granulocytes, while the

periphery around the capsule of the liver appeared to contain some lining connective tissue fibers, as noted in Fig. 4. The central vein in the hepatic had congestion and blood granules poured into it, which appeared in the form of a wide network of channels around the rows of cells, as noted in Figure 5. These effects were similar to what was obtained Al-Hadithi [48], and these changes in the liver were also observed by Martínez et al. [49]. Nauciel et al. [50] stated that oral infection with *S. typhimurium* spread rapidly in the intestine possibly through Peyer's patches from the intestinal-associated lymphoid tissue to the reticuloendothelial (RES) organs including the liver and spleen, and bacterial cysts may be due to an increase in bacterial virulence factors [51].

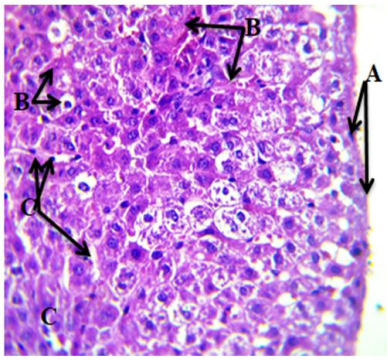

FIGURE 3. Liver tissue, hepatocyte hypertrophy (A) granulocytes with kupffer cells (B) erythrocytes, bile duct (C) portal vein branch with blood clot (D) infiltration of white blood cells in the portal area (E) CH 2 EY 40

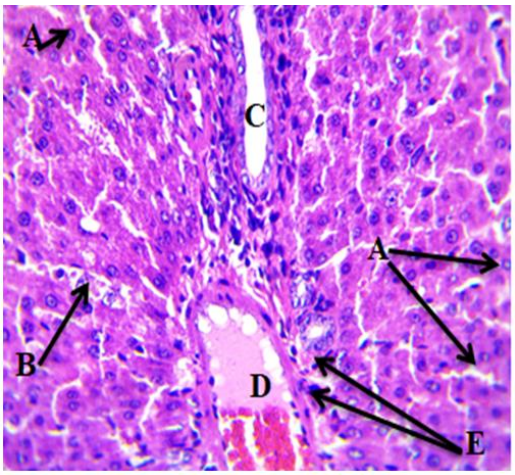

FIGURE 4. Peripheral tissue of the liver capsule (A) composed of connective tissue paved, hepatocytes enlarged and their nuclei thickened (B) kupffer cells in granulocytes (C) CH 2 EY 40

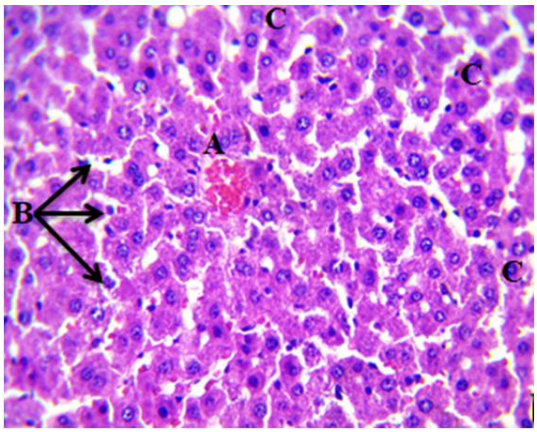

FIGURE 5. Hematopoietic congestion in the central vein (A) The network of granulocytes and which contains kupffer cells (B) Hepatocyte rows (C) CH 2 EY 40

The spleen tissue contained a wide lymphatic nodular assembly represented in the white pulp and in the middle was the nodular artery or the white pulp artery with a wide spread of lymphocytes around the nodular assembly, where the splenic pockets were also filled with blood, as noted in Fig. 6, while some blood cells appeared in the spleen tissue erythrocytes, lymphocytes, and the rest of the other leukocytes and macrophages containing hemosiderin pigments are dark in color, as noted in Fig. 7. As for the splenic pockets filled with red pulp, they were filled with erythrocytes, where hyperemia appeared in those pockets loaded with white blood cells as well, in addition to the presence of lymphatic tissue it consisted of bundles of bile fibers supporting the spleen board, as noted in Fig. 8, and the surroundings of the spleen tissue contained necrosis in the connective tissue and caves that contained the debris of some damaged cells and the presence of a number of macrophages in the splenic blood pockets containing erythrocytes and leukocytes as well as adjacent to the lymphatic nodules represented by white pulp, as noted in Fig. 9. Some of these lesions were reported Al-Zoury [52] in experimentally infected guinea pigs with *S. typhimurium*.

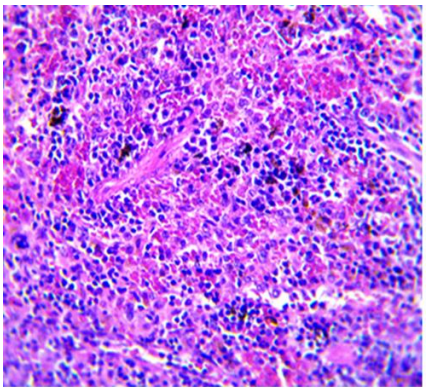

FIGURE 6. Spleen tissue, white pulp-forming lymph nodule (A) Nodular artery (B) Splenic sinus filled with blood and scattered lymphocytes (C) CH 2 EY 40

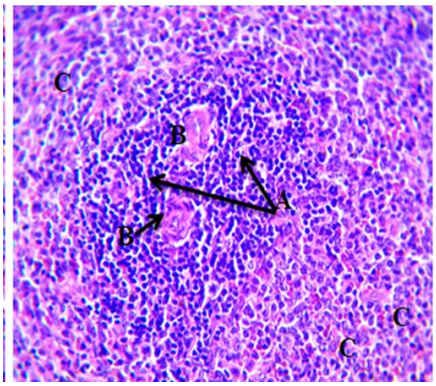

FIGURE 7. Spleen parenchyma, lymphocyte pool with erythrocytes, presence of macrophages and hemosiderin(B) CH 2 EY 40

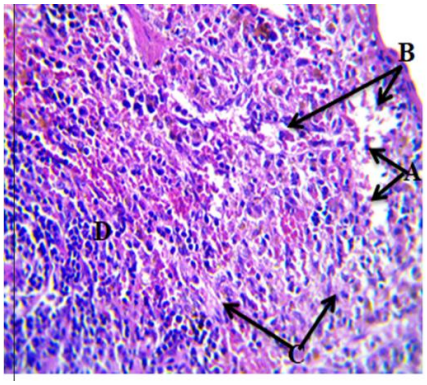

FIGURE 8. Splenic sinuses with hyperemia and infiltration of large numbers of leukocytes and other lymphocytes (A) Spleen fibrous sacs (B) CH 2 EY 40

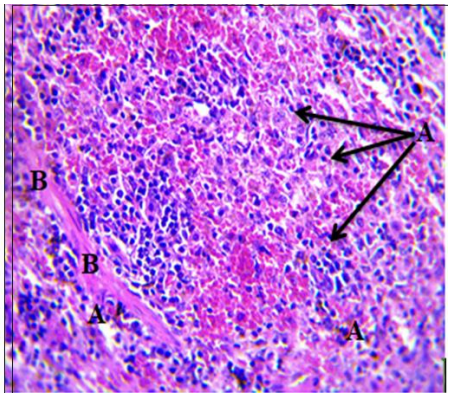

FIGURE 9. Necrosis of the spleen tissue and the presence of cavitation and vacuoles in it (A) Damaged cells in the cavernous (B) Splenic pockets filled with blood (C) Lymph nodular pool (D) CH 2 EY 4

In the kidney, glomeruli appeared in its cortex, with erosions, the spread of leukocytes on the surface of the glomeruli, and their access to the dilated capsular space surrounded by Bowman's capsule. Most of the nearby convoluted and gastric tubules expand in their lumen and degeneration of cells lining the walls of these tubules, in addition to the presence of blood congestion in the blood vessels around the glomerulus, as noted in Fig. 10. As for the glomeruli of the kidneys, they appeared to be lobed with rocks in the middle of the lumen of Bowman's capsule, and some cavities of the convoluted tubules had scaly epithelial cells with congestion of the blood vessel surrounding the glomeruli, as noted in Fig. 11, and the kidney pulp contained inflammatory edema and homogeneously dissolved blood in the interstitial tissue with congestion of most of the capillary blood vessels in the tissue, as well as the presence of a proliferation of numbers of white blood cells and macrophages between the renal tubules containing a limited number of scaly epithelial cells, as noted in Fig. 12. These results are close to what Al-Hashemi [53] in mice infected with *S. enteritidis*.

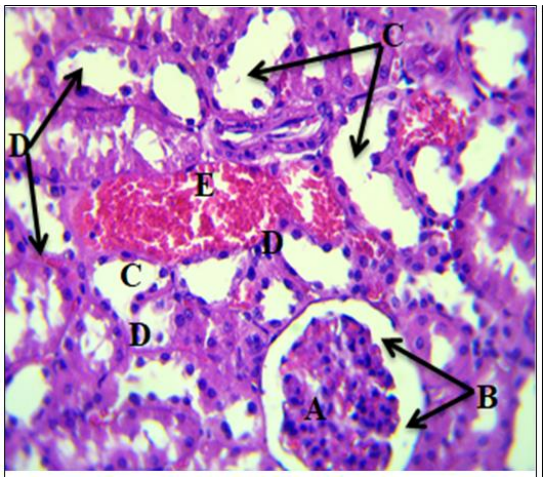

FIGURE 10. Kidney cortex, glomerulus atrophy (A) capsular space dilatation (B) dilatation of the lumen of convoluted granules (C) and degeneration of its cells lining the lumen (D) Periglomerular congestion (E) CH 2 EY 40

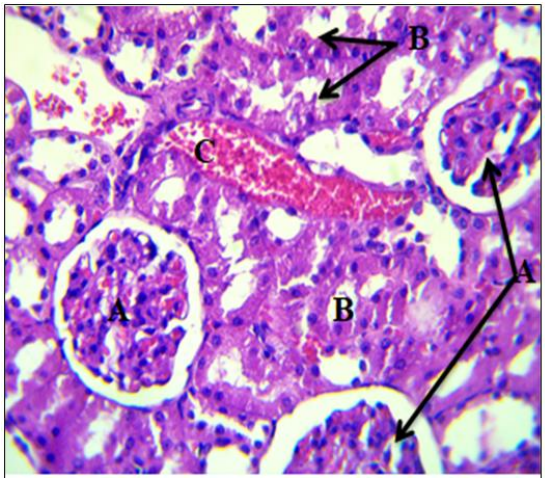

FIGURE 11. anatomy and lobed glomeruli (A) Epithelial cell necrosis (B) Tubule lumen, periglomerular congestion (C) CH 2 EY 40

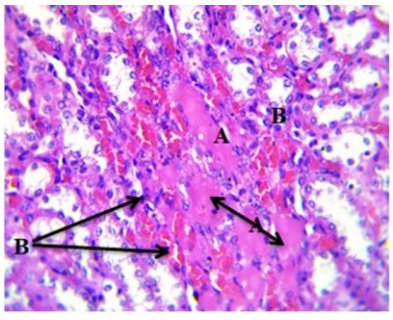

FIGURE 12. Kidney medulla, inflammatory edema, hemolysis blood (A) in the interstitial, leukocytes and macrophages (B) between the tubules CH 2 EY 40

Decomposed blood appeared in the cavity of the heart, with the disappearance of the endothelial cells, as well as the basement membrane based on those cells from the

endocardial layer, and the heart muscle appeared in the form of bundles of cardiac muscle fibers stacked with each other, as noted in Fig. 13, while the longitudinal bundles of cardiac muscle fibers appeared it contained some leukocytes with a fascia of muscle fibers with a noticeable disintegration between the muscle fiber bundles of the outer part of the muscle of the heart wall, as noted in Fig. 14, and there was extensive blood congestion of the branches of coronary blood vessels between the bundles of cardiac muscle fibers and the spread of a number of white blood cells around it, as noted in Fig. 15, and this result differed with what Al-Hadithi [48] in mice of infiltration of inflammatory cells around the blood vessels engorged with cardiac muscle fibers.

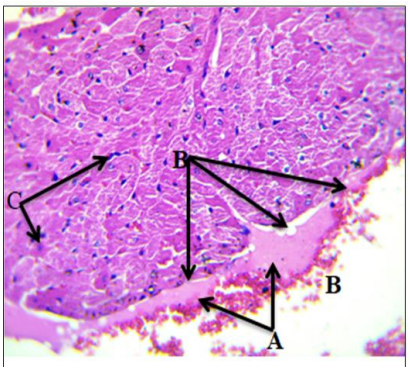

FIGURE 13. Hemolytic blood in the cavities of the heart (A) Disappearance of endothelial cells and the basement membrane on which cells are based (B) In the endocardial region, compact cardiac muscle bundles (C) CH 2 EY 40

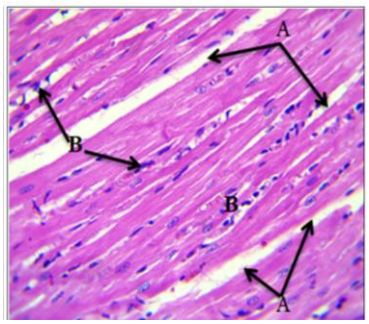

FIGURE 14. Dissociation of cardiac myofibrils (A) leukocytes (B) of myofibrils with macrophages (C) CH 2 EY 40

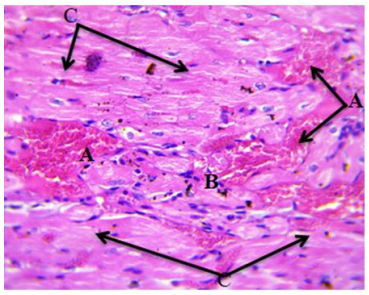

FIGURE 15. Extensive blood vessel congestion (A) coronary area, infiltration of some leukocytes (B) cardiac muscle fibers (C) CH 2 EY 40

While the infiltration of large numbers of inflammatory leukocytes surrounding the alveolar walls appeared in the interstitial tissue of the lung, in addition to the presence of homogeneous inflammatory edema and dissolved blood in the interstitial tissue with thickening of the walls of the alveoli composed of colloidal fibers, as noted in Fig. 16, As for the epithelial cells lining the bronchi in the lung, they showed degeneration and sloughing of some cells, and the muscle layer surrounding the bronchi had infiltration of numbers of inflammatory leukocytes scattered in the interstitial tissue lining around the alveoli, in which some blood cells appeared in its lumen, as noted in Fig. 17, our results agreed with what was reached AL-joboury [54] in mice and guinea pigs infected with *S.typhi*.

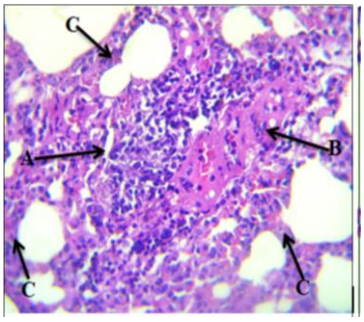

FIGURE 16. Lung tissue with infiltration of white blood cells (A) around the alveoli, homogeneous inflammatory edema (B) thickening of the alveolar wall (C) CH 2 EY 40

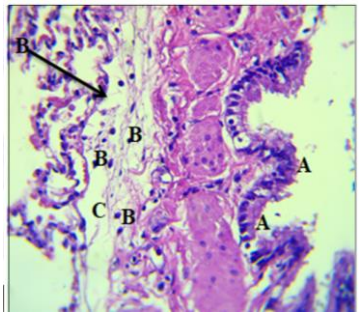

FIGURE 17. Degeneration and necrosis of bronchioles and lung cells (A) leukocytes infiltration (B) into the interstitial tissue (C) CH 2 EY 40

Liu et al. [55] stated that flagella are necessary for the invasion of host epithelial cells, and in Olsen et al. [4] also indicated the necessity of flagella in infecting epithelial cells, which results in a toxic effect. *S. typhimurium* flagella are expressed within epithelial cells and can appear in cultured HeLa cells infected [56]. During *S. typhimurium* invasion of the body, TLR-5 stimulation by flagella causes an inducible inflammatory immune response, and this occurs within 15 minutes [57], and this invasion can be attributed to several systems that control both flagella and gene expression of virulence [58], while Schmitt et al. [59] indicated that flagella are necessary to initiate inflammation, creating an environment in which *S. typhimurium* predominates.

4. Conclusions

The flagellar antigens of *S. typhimurium* showed high immunization activity in the experimental rats and this was evident in the clinical signs during the experiment, while the histological study showed the appearance of lesions and necrosis in various organs, which indicates the high immune response caused by these flagella in addition to their toxic effect, which was evident in the

degenerations and dissections that were seen on the organs of the rats under study, so it is recommended to conduct extensive studies on the immunological and toxic effect of flagellated antigens and reduce them.

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