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Original Research Article

Histological and Physiological Evaluation of *Salmonella Spp.* Pathogenicity Experimentally Dosed in Male Albino Mice

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Abstract: The research aimed to isolate and diagnose the serological strains of *Salmonella spp.* that cause typhoid fever, as well as those that cause intestinal inflammations, and to study their histological pathogenesis after experimentally dosed in immunosuppressed male albino mice. The research included 180 blood and stool samples from people with typhoid fever, and 20 samples from people with diarrhea, as well as 30 samples representing the control group. The bacteriological results showed that 26 patients were diagnosed with *S. typhi* out of 180 patients with typhoid fever, representing 14.4%, and 5 patients were diagnosed with *S. typhimurium* out of 20 patients with diarrhea, representing 25%. The results of the study showed an increase in the levels of liver enzymes, as the average concentration of the ALT enzyme for the category of patients infected with typhoid, with a recent and confirmed infection with *S. typhi*, reached 11.283 international units/liter, and the average concentration of the AST enzyme for the same category reached 20.1 international units/liter, compared to the control group. 48 male laboratory mice were used and distributed into 4 groups of 12 mice per group. The first group was the control group that was dosed with physiological solution in an volume of 0.2 ml. The second group was dosed with a suspension of *S. typhi* bacteria in a volume of 0.2 ml and a concentration of 1×108 colonies/ml. The third group was dosed for a week with the immunosuppressant cyclosporine before and after the experimental infection with a suspension of *S. typhi* bacteria in a volume of 0.2 ml and a concentration of 1×108 colony/ml. The fourth group was dosed with a suspension of *S. typhimurium* bacteria in a volume of 0.2 ml and a concentration of 1×107 colony/ml. The mice were dissected one week, two weeks and three weeks after the experimental infection, at a rate of 3 mice per week from each group. The blood was collected and the liver was removed from each mouse. The results showed a slight increase in the ALT enzyme average concentration for the group of mice treated with the immunosuppressant cyclosporine and dosed with *S. typhi*, reaching 18.57 IU/L, while the group of mice dosed with *S. typhimurium* was within the limits of the control group and reached 12.24 IU/L. The AST enzyme concentration rate in the control group was 15.136 IU/L, and for the group of mice treated with the immunosuppressant cyclosporine and dosed with *S. typhi*, it reached 24.74 IU/L, while the concentration for the group of mice dosed with *S. typhimurium* reached 21.76 IU/L. As for the histological study, the results of the microscopic examination showed the occurrence of many histological lesions in the liver, represented by severe degeneration and acute necrosis, with hypertrophy and sometimes hyperplasia in the hepatocytes, and the loss of chromatin in the nuclei of a number of them, with thickening of the nuclear envelope and sometimes thickening of the nuclei, as well as vacuolation of the cytoplasm in other hepatocytes, with an increase in the number and size of Kupffer cells in the blood sinusoids, which varied in their appearance between narrowed, dilated and congested with blood. It was found that these pathological lesions were more severe in the group of mice treated with the immunosuppressant cyclosporine and dosed with *S. typhi*.

Keywords: Typhoid fever, cyclosporine, *Salmonella spp.* pathogenesis, histological effect of *Salmonella spp*.

1. INTRODUCTION

Typhoid fever, caused *by Salmonella enterica serovar typhi*, infects more than 20 million people and kills nearly 200,000 people annually, mostly in developing countries. The bacteria is transmitted through contaminated food or water. *S. typhi* bacteria are distinguished from other *Salmonella* species and other genera of the Enterobacteriaceae family by their special structures, as they possess a virulence antigen (Vi-Ag) that increases their virulence, as well as their ability to secrete a distinctive toxin [1]. *Salmonella enterica serovar typhimurium* behaves differently in its pathogenesis, as it causes local enterocolitis in humans, but when infected with mice, it behaves similarly to the pathogenesis of typhoid fever in humans. This difference has given some concepts of the pathogenesis of *S. typhi*, but to a limited extent due to the existence of some differences between them. Diarrhea is the main clinical feature of Salmonellosis, and the incubation period ranges from five hours to seven days, but clinical signs usually begin 12 to 36 hours after ingestion of food or water contaminated with *S. typhimurium*, while *S. typhi*, which is responsible for typhoid fever, begins with high temperatures during the incubation period, which extends from 5-21 days, as temperature is a distinctive sign of the disease, and is found in more than 80% of infected people [2]. It is obvious to notice severe tissue damage and lesions in the hepatocytes, as the liver is the organ in which many substances entering the body are metabolized and detoxified in one way or another. Liver is responsible for removing detoxification, through which the body gets rid of the largest possible amount of toxic substances by breaking down unwanted substances. The most measured liver enzymes are Alanine transaminase (ALT) and Aspartate transaminase (AST), which provide important indicators of its safety [3]. The arrival of *S. typhi* to the liver during infection warns of a future danger, as it can easily move to the gallbladder, and its clinical signs gradually diminish, and the patient turns from an acute disease to a chronic carrier state, and thus the bacteria spread greatly. Therefore, it is necessary to take the appropriate treatment for it and perform a liver enzyme test periodically during infection until the end of the treatment period [4, 5].

2. MATERIALS AND METHODS

2-1 Culture media, isolation and bacterial diagnosis

200 samples were examined, aged between (17-65) years and of both sexes, while the control group consisted of 30 samples. Stool and blood samples were taken from the individuals who showed clinical signs, and the samples were transferred directly to the laboratory to perform the remaining required laboratory tests. MacConkey Agar and X.L.D. Agar were used for bacterial culture, while S.S. Agar was used to confirm their characteristics and to obtain pure and single colonies, as well as the heart and brain infusion agar, then biochemical tests were performed to confirm their diagnosis, where VITEK 2 was used to enhance the confirmation of the isolates' diagnosis process up to the species level [6].

2-2 Determination of the lethal dose–50 (LD-50)

The lethal dose–50 was determinated according to Al-Aarajy, it was the fourth sequence in dilution at a concentration of 1×108 colony/ml for *S. typhi*, and the fifth sequence in dilution at a concentration of 1×107 colony/ml for *S. typhimurium* [7].

2-3 Experimental infection of male mice with *Salmonella* enterica

48 male Balb/c mice, aged (8-12) weeks, weighing between (22-28) g, were used and randomly distributed into four groups, with 12 mice in each group, as follows:

1. Group 1 (control group): treated with physiological solution in an amount of 0.2 ml once a day for a week.
2. Group 2: treated with *S. typhimurium* bacteria in a volume of 0.2 ml once 1×107 colony/ml.
3. Group 3: treated with *S. typhi* bacteria in a volume of 0.2 ml once 1×108 colony/ml.
4. Group 4: treated with the immunosuppressant Cyclosporine for a week, then dosed with *S. typhi* bacteria once, then treated with the inhibitor Cyclosporine for another week.

After confirming the infection, the mice were dissected weekly, three mice from each group, over a period of three weeks. Glass slides of liver tissue are then made via the sectioning method depending on the traditional method, which includes fixation, washing, dehydration, clearing, infiltration, embedding, trimming and sectioning, staining, and mounting. Then the tissue sections were examined and photographed under a light microscope at different magnification powers, as well as to measuring the liver enzymes Alanine amino transferase (ALT) and Aspartate amino transferase (AST) in the blood serum of the mice.

3. RESULTS AND DISCUSSION

3-1 Isolation and identification of *Salmonella*

The results of laboratory culture showed 26 patients with typhoid fever out of a total of 180, as shown in Table (3-1), and *Salmonella* appeared on X.L.D. agar in the form of small, smooth, round, red colonies with a black center, and on MaCconkey agar, smooth, colorless, non-lactose fermenting. The determination of all *Salmonella* isolates up to the strain under investigation was then confirmed using the VITEK 2 diagnostic system. MaCconkey agar is a differential and selective medium for Gram-negative bacteria, while XLD agar and S.S. agar are highly selective and designed to prevent

the growth of most types of *E. coli*, because they contain sodium thiosulfate, which allows *Salmonella* to develop among clinical types [8, 9].

Table (3-1): *Salmonella* infections percentages

Total samples: 230 (100)%						
Total samples positive for *Salmonella* isolates: 31 (13.47)%						
T	**Category**	**the number**	**The ratio**	**The number, percentage and type of positive laboratory culture swabs**		
				the number	**The ratio%**	**Positive isolation type**
1	People with typhoid	180	78.26%	26	14.4%	*S. typhi*
2	People with diarrhea	20	8.7%	5	25%	*S. typhimurium*
3	Control group	30	13.04%	0	0%	-
	The total number	230	100%	* Pearson Chi-Square = 7.094 P-Value = 0.029		

Accurate diagnosis of typhoid fever at an early stage is important, and this importance comes not only from the point of view of diagnosing the pathogen but also to determine people who may be carriers of it, who have an effective role in the outbreak of acute typhoid fever in the community in which they live. The results of the rapid typhoid IgG / IgM test showed significant differences at the probability level of P-Value = 0.005, as 134 infected people, at a rate of 74.4%, showed a positive result for the antibody IgM+ only, which indicates a recent infection. Among them, 26 infected people showed a positive result for the stool culture for the type *S. typhi*, while the rest of the test categories were negative for the stool culture, while 4.4% were positive for the antibody IgG+, which indicates a previous infection with the disease, and 21.2% were positive for IgM+ and IgG+ together, which indicates an old and recent infection or an old infection and not recovered, while the control group was negative for both IgM- and IgG-, as the living body, after the immune response to the pathogen, begins to secrete the IgM antibody, the concentration of which gradually decreases inside the body, and then the body begins to secrete the IgG type, the concentration of which remains for several months. This test is one of the important serological tests used in laboratories, and it is of great importance in identifying patients with typhoid fever. Since the control group in our research was negative for the test at a rate of 100%, it gives high sensitivity in confirming the absence of infection for people who have symptoms similar to typhoid fever.

The sensitivity of the test to positivity is limited. The results of the study showed that only 26 patients (14.4%) of those infected with typhoid had a positive laboratory culture result for *S. typhi*. These results are consistent with what Farhan found, as the infection rate was 13.3%. They showed that serological methods are less effective in the laboratory diagnosis of typhoid fever. The infection rate was 15% in the results of Hassouni, who indicated that the test is good for differentiating between typhoid fever and other diseases with similar clinical signs [10, 11]. The low sensitivity of this test may be attributed to the usual exposure of individuals in the community to *Salmonella* antigens through contaminated water and food. Although these subclinical doses of *Salmonella* are incapable of causing overt clinical disease, they continually enhance the host's immune system, leading to the cumulative presence of these antibodies in the blood circulation. *Salmonella* infection may also lead to a broad, polyclonal stimulation of the immune response, resulting not only in specific antibodies to it, but also in antibodies to a reactive group or other groups of its O and H antigens. This may explain the multifaceted picture of serological results that can often be encountered during the serological diagnostic evaluation of *Salmonella* infection [12].

Laboratory culture remains one of the best and most accurate methods used to diagnose *Salmonella*. The sensitivity of the test varies according to the skill of the technician and the type of sample used in laboratory culture. Blood is less sensitive to S. typhi than stool. In this study, we were unable to isolate it from the blood. The main reason for this is that it is only present in the blood during the first week of infection. The same is the case with traditional methods of diagnosing it and giving antibiotics, because its numbers are low in the blood [13].

Stool is a good example for isolating most types of *Salmonella*, as the infected person begins to shed S. typhi in the stool from the end of the first week to the end of the third week, while in the chronic case it can continue to shed it throughout his life. In its isolation, care must be taken to take a good amount of stool and culture it for more than one replicate of the same sample, and the sample must be taken again if the sample is negative to ensure that the infected person is free of it, because its shedding in the stool is intermittent and not continuous. The researcher [14] was unable to isolate it from the blood, but he was able to isolate it from the stool, while the researcher [15] was able to isolate it from the blood and the stool. It can also be isolated from the rest of the body fluids, as it was possible to isolate it from bile (its favorite place to hide) [16], while one isolate of it was isolated from urine in the third week of infection [7].

3-2 Assessment of Liver Enzymes Efficiency

3-2-1 Assessment of the activity of liver enzymes ALT and AST in humans

The results of the basic values of ALT enzyme absorption in human serum showed significant differences at the probability level of P-Value = 0.049, as its average concentration in the category of patients infected with typhoid with recent and confirmed infection with *S. typhi* (TMM) reached 11.283 IU/L, which is higher than its concentration in the control group. Its average concentration rate also increased for the category of patients infected with typhoid with recent and unconfirmed infection (TM) and the category of patients infected with typhoid with previous infection and negative in laboratory culture (TMG) than the normal average for the control category. While the results of the basic values of the absorption of the AST enzyme in human serum showed significant differences at the probability level of P-Value = 0.015, as the average concentration of the enzyme in the TMM category reached 20.10 IU/L, while in the TM and TMG categories it reached (14.39, 22.34) IU/L, respectively, which means that it is higher than the normal average in the control category, which reached 11.10 IU/L, as shown in Table (3-2).

Table (3-2): ALT and AST enzyme results in humans for groups infected with typhoid

T	Studied categories	Number of infected people For each category n(%)	ALT concentration international unit/liter (U/I) standard deviation (Standard deviation) ± Average (Mean)	AST concentration international unit/liter (U/I) standard deviation (Standard deviation) ± Average (Mean)
1	TMM	6 (14.3%)	11.283 a ± 2.227	20.10a±6.91
2	TM	24 (57.1%)	9.371 b ± 2.651	14.39 b±7.04
3	TMG	8(.119%)	10.130 ab±3.81	22.34a±8.08
4	Control group	4 (9.5%)	6.130 c±2.03	11.10b±1.27
	the total	42(100%)	* P-Value = 0.049	** P-Value=0.015

TMM = patients with typhoid fever with recent confirmed *S. typhi* infection, TM = patients with recent culture-negative typhoid fever, TMG = patients with previous culture-negative typhoid fever, * = significant differences, similar letters mean no significant differences, ** = significant differences, similar letters mean no significant differences.

The liver is one of the most important organs in the body and has many and varied functions, perhaps the most important of which is cleaning the body of toxins and dangerous substances, as well as resisting many causes that lead to diseases, as it is part of the immune system. Therefore, the dysfunction that affects hepatocytes as a result of infection with pathogens leads to their damage and the leakage of cell contents into the bloodstream, and thus an increase in the level of its enzymes above the normal limit. Our results agreed with what other researchers have reached, that there is a significant increase in the rate of liver enzymes ALT and AST, reaching (31.4 and 26.8) international units/liter, respectively, in patients infected with typhoid and diagnosed only by serology [17]. Another study showed that typhoid fever leads to a significant increase in liver enzymes, and that this is due to infection with the endotoxins of *Salmonella typhi*, noting that the other internal organs of patients were not targeted by this type of *Salmonella* [18]. In another study, among the children with jaundice who were hospitalized, there were those who showed a positive result for typhoid testing, and infection with the *S. typhi* type was confirmed in them by laboratory culture, and it was the main cause of damage to some parts of their liver [19]. The results of another detailed study showed that there is a significant increase in the levels of ALT and AST enzymes in typhoid fever patients. In the same context, the results of the study reduced the importance of the two enzymes as a diagnostic tool for this fever because the AST enzyme is not only found in liver tissues but is also found in many other human tissues, especially the heart and skeletal muscles, while the ALT enzyme is more important because its presence outside liver tissues is less, and liver enzymes quickly return to normal and there is no requirement for a pathological cause to change their concentration because many factors affect their concentration in human blood [20].

3-2-2 Assessment of the activity of liver enzymes ALT and AST in mice

The results of the basic values of the absorption of the enzymes ALT and AST in the serum of mice showed no significant differences between the categories at the probability level of P-Value = 0.051, as the average concentration of ALT in the control group was 11.43 IU/L, and in the group of mice dosed with *S. typhi* (MT) 13.41 IU/L. The results also showed a slight increase in the group of mice treated with the immunosuppressant cyclosporine and dosed with *S. typhi* (MTC) reaching 18.57 IU/L. As for the results of the AST enzyme test, they showed no significant differences between the categories at the probability level of P-Value = 0.051, as its average concentration in the control group was 15.136 IU/L, and in the group of mice treated with the immunosuppressant cyclosporine and dosed with *S. typhi* (MTC), it reached 24.74 IU/L, as shown in Figure (3-1).

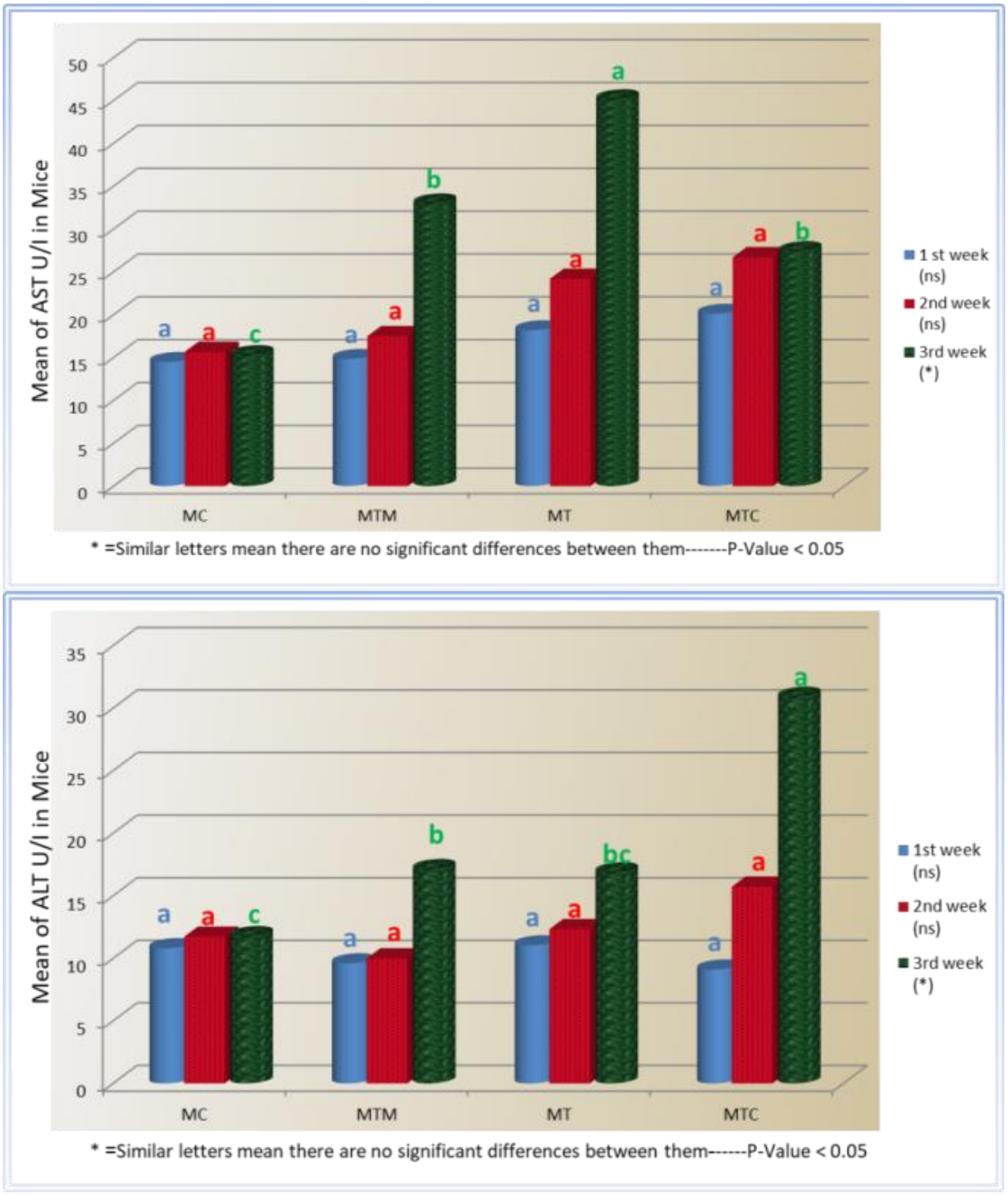

Figure (3-1):
MT = mice treated with *S. typhi*, MTC = mice treated with cyclosporine and treated with *S. typhi*, MTM = mice treated with *S. typhimurium*, MC = control mice, W = week, ns = no significant difference, similar letters mean no significant difference between them.

The results of a study showed that the average values of the control group of mice were 12.8 IU/L for ALT and 24.5 IU/L for AST, and their results were close to what we reached [21], while other researchers reported that the average values of the control group of mice were 33.5 IU/L for ALT and 60.0 IU/L for AST. The normal values of liver enzymes in healthy mice are not agreed upon and depend on the experimental conditions, so the presence of a control group in each experiment is necessary to evaluate its results, and companies specialized in manufacturing test solutions give different values according to the working conditions under which those solutions were manufactured [22]. The results of a study conducted at the Institute of Molecular Nutrition in China showed that the levels of ALT and AST enzymes in mice were higher than the normal average of the control group, and that the increase occurred after *S. typhimurium* succeeded in crossing the intestinal barrier to reach the liver, as proven by the histological changes that occurred in it. In the same study, therapeutic methods were adopted through which a decrease in the concentrations of ALT and AST in the liver appeared, and its results were identical to what was reached in our research, as the absence of significant differences in the first and second weeks means a logical explanation for the failure of *Salmonella* to reach the liver or it reached it but needed more

time to be able to do so, which negatively affects the concentration of its enzymes [23]. Figure (3-1) shows a significant increase in the ALT enzyme in the MTC group at the third week of infection, and this may indicate our success in infecting mice with *S. typhi*, especially since it is reinforced by histological evidence of infection of the mouse liver in its third week. Other researchers have been able to infect Wistar rats with *S. typhi*, and liver enzymes have been elevated as a result of its leakage into the bloodstream due to liver damage demonstrated by histological sections [24]. Our inability to isolate *S. typhi* from stool, blood, or urine brings us back to the same problem of the lack of a sensitive animal model for *S. typhi*. Although laboratory mice are the closest model that can be worked on, Tsolis published a wonderful article on the role of methods and techniques in making animals sensitive to *S. typhi*. They emphasized in their article that each animal model has shortcomings that limit its usefulness in studying some aspects of the disease associated with *Salmonella* serotypes, so we must think carefully before making a decision about the animal model, because any change in the animal will negatively affect our understanding of its pathogenesis. It is likely that animal models for *S. typhi* will continue to be developed in the future, and the focus will mostly be on mice [25].

4- Histological examination

Histological examination of the mice of the first group showed the normal structure of the liver visceral tissue, consisting of cords or hepatic plates formed by polygonal epithelial hepatocytes. The distinctive shape of the hepatocytes was also observed in a radial arrangement around the central vein, separated by blood spaces representing sinusoids containing macrophage Kupffer cells, as in Figure (3-2).

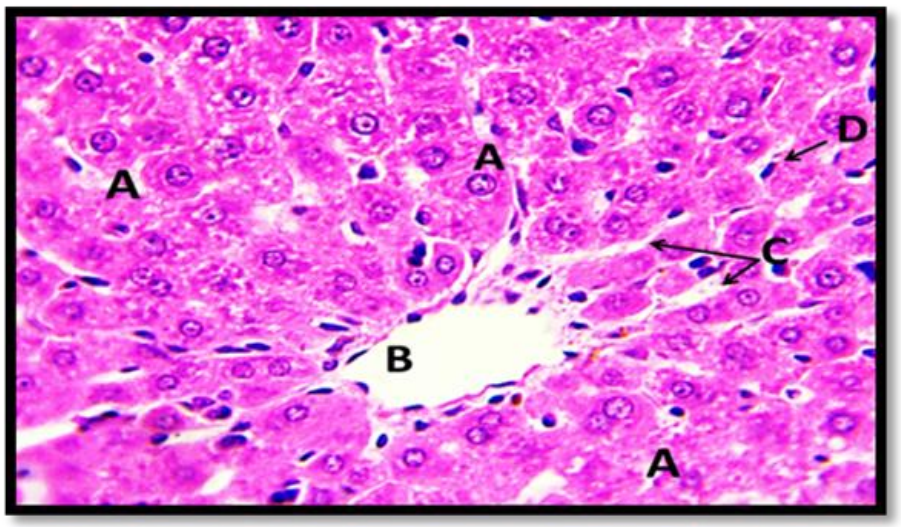

Figure (3-2): Micrograph of the mouse liver of a control group, showing hepatocytes arranged radially (A) around the central vein (B) separated by sinusoids (C) containing Kupffer cells (D). (H&E, X40)

The second group treated with *S. typhimurium* bacteria showed degeneration, necrosis, hypertrophy in the hepatocyts, pyknotic nuclei with vacuolation in the cytoplasm and absence of pigment in most of its areas, as well as congestion of the central vein in the center of the hepatic lobule and partial desquamation of the endothelial cells, with acute hyperemia in the portal vein and thickening of its wall, furthermore infiltration of inflammatory cells around it and around the bile duct and the branch of the hepatic artery. It was also noted that there were many Kupffer cells and their enlargement, and dilated sinusoids, as in Figures (3-3), (3-4), (3-5), (3-6), (3-7), (3-8).

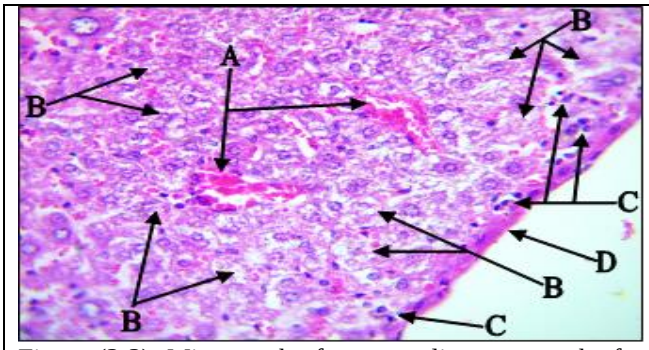

Figure (3-3): Micrograph of a mouse liver one week after experimental infection with *S. typhimurium* bacteria, showing hemorrhage (A), degeneration and acute necrosis

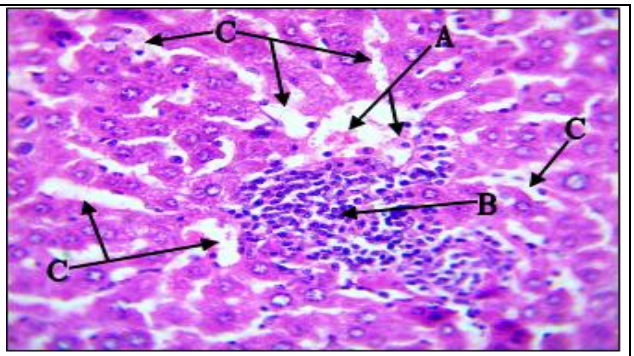

Figure (3-4): Micrograph of a mouse liver one week after experimental infection with *S. typhimurium* bacteria,

of hepatocytes and unclear borders, as well as vacuolation of the cytoplasm of most of it (B), infiltration of inflammatory cells (C), and appearance of part of the liver capsule (D). (H&E, X40).

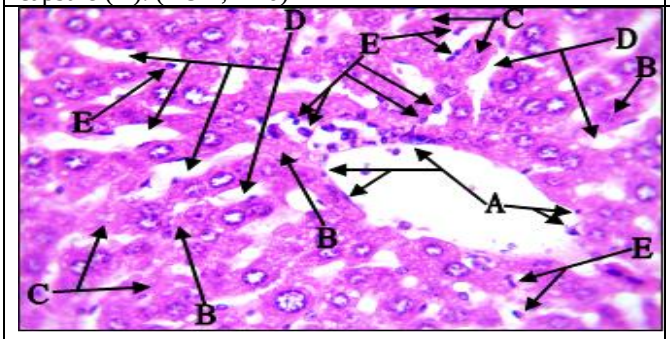

Figure (3-5): Micrograph of a mouse liver two weeks after experimental infection with *S. typhimurium* bacteria, showing inflammatory cells in the walls of the central vein (A), hepatocyte degeneration (B) and necrosis (C), dilatation of the blood sinusoids (D), and an increase in the number and hypertrophy of Kupffer cells (E). (H&E, X40).

showing partial blood congestion in the central vein (A), acute focal clustering of inflammatory cells (B), dilated sinusoids (C), and unclear cell borders throughout the tissue section (H&E, X40).

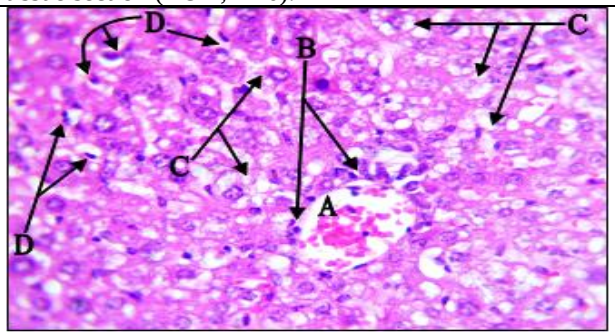

Figure (3-6): Micrograph of a mouse liver two weeks after experimental infection with *S. typhimurium* bacteria, showing thrombus in the central vein (A), infiltration of inflammatory cells around it (B), general degeneration and necrosis of most of the hepatocytes, with vacuolar degeneration of a number of them (C), and hypertrophy of Kupffer cells (D). (H&E, X40).

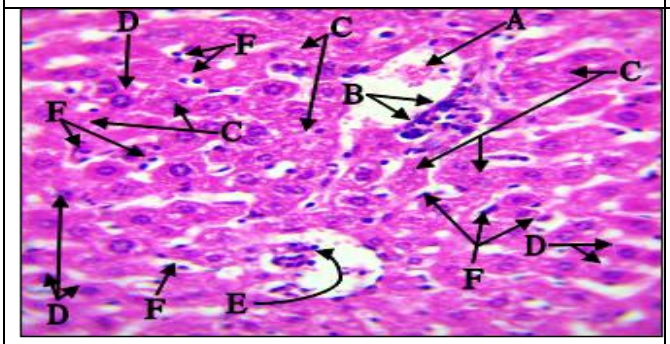

Figure (3-7): Micrograph of a mouse liver three weeks after experimental infection with *S. typhimurium* bacteria, showing partial blood congestion in the central vein (A), with a dense clustering of inflammatory cells on one side (B), acute degeneration and necrosis in a number of hepatocytes (C), and thickening of the nuclei of a number of others (D), dilatation of one of the sinusoids and its partial congestion with blood, with an clustering of inflammatory cells in its cavity (E), and an increase in the number of Kupffer cells (F). (H&E, X40).

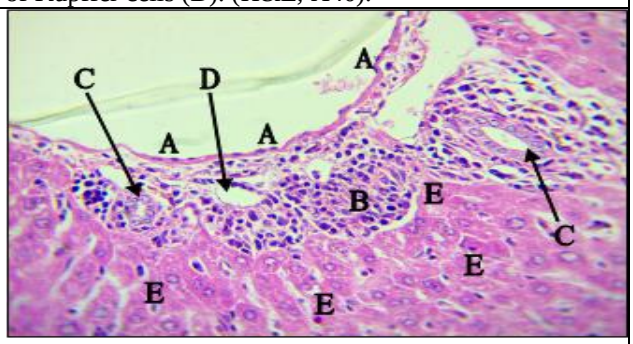

Figure (3-8): Micrograph of a mouse liver three weeks after experimental infection with *S. typhimurium* bacteria, showing the portal vein surrounded by a dense infiltration of inflammatory cells (A), as well as their focal clustering below it (B), which extended to surround both branches of the bile duct (C), and the branch of the hepatic artery (D), degeneration of the hepatocytes and loss of their characteristic shape (E). (H&E, X40).

Invasive bacteria cause severe tissue lesions in the liver as a result of their direct effects or toxins on hepatocytes, or indirect effects represented by stimulating the immune system. When *S. typhimurium* invades, inflammatory cells aggregate, causing inflammation, which is a vital process initiated by the immune system in response to tissue injury caused by bacterial infection and other harmful stimuli. The acute inflammatory response is characterized by dilation of blood vessels, diapedesis of white blood cells to the site of injury to destroy the invading pathogens, and an increase and activity in the secretion of specialized cytokines, followed by a rapid resolution and repair phase of damaged tissues [26]. Several researchers have reported that the liver was the main target organ for infection, and *S. typhimurium* was highly virulent to it [27], while other researchers have indicated that infection with *S. typhimurium* bacteria causes a systemic infection in mice that is different from its infection in humans, as it causes intestinal infections. They also reported that it begins in mice through absorption and secretion of certain proteins, and after absorption, it is found inside compartments in SCV cells, which is similar to *S. typhi* in humans [28].

The results of the third group treated with *S. typhi* bacteria showed severe degeneration and necrosis, with hypertrophy and sometimes hyperplasia in the hepatocytes, loss of chromatin in the nuclei of some of them, with thickening of the nuclear envelope and sometimes thickening of the nuclei, as well as the vacuolation of the cytoplasm in other hepatocytes, damage to the wall of the central vein and sometimes a change in its shape and congestion, furthermore the

infiltration of inflammatory cells near it or in scattered areas of the tissue parenchyma in a focal manner, with an increase in the number and size of Kupffer cells in the blood sinusoids, as well as the occurrence of hemorrhage and sometimes inflammatory edema in the tissue parenchyma that lost its distinctive features as a result of the scattering and degeneration of its cells and their severe necrosis, as in Figures (3-9), (3-10), (3-11), (3-12), (3-13), (3-14).

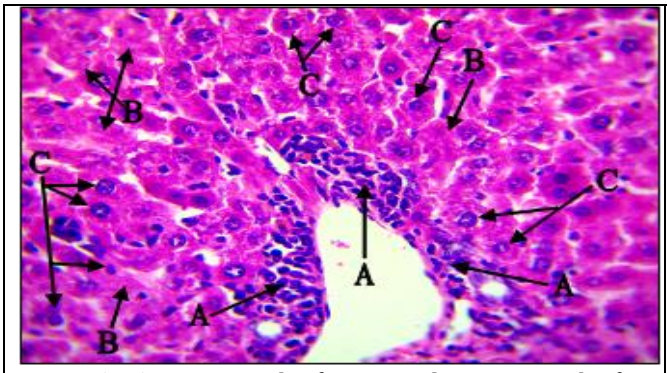

Figure (3-9): Micrograph of a mouse liver one week after experimental infection with *S. typhi* bacteria, showing the central vein surrounded by a dense infiltration of inflammatory cells (A), degeneration and necrosis of a number of hepatocytes (B), thickening of the nuclei of a number of others (C), and an increase in the number and size of Kupffer cells in all of the blood sinusoids in the tissue section. (H&E, X40).

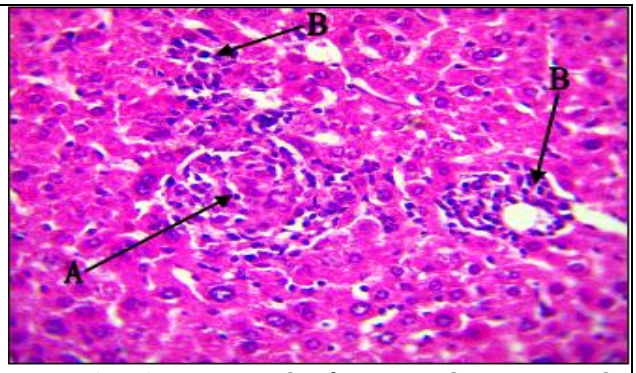

Figure (3-10): Micrograph of a mouse liver one week after experimental infection with *S. typhi* bacteria, showing a focus of inflammatory cells surrounding degenerated hepatocytes (A), dense infiltration of inflammatory cells in scattered areas of the liver parenchyma (B), and acute degeneration and necrosis of hepatocytes throughout the tissue section, leading to the disappearance of its distinctive features (H&E, X40).

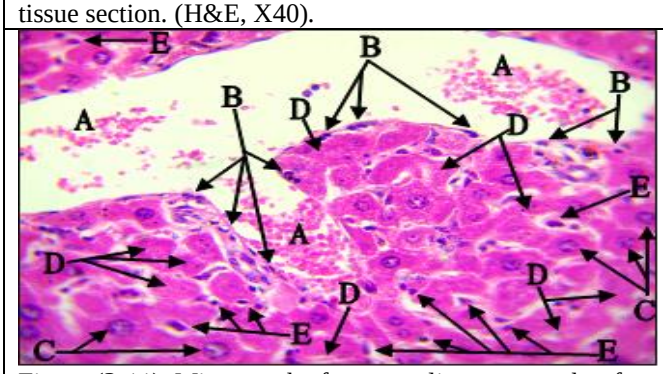

Figure (3-11): Micrograph of a mouse liver two weeks after experimental infection with *S. typhi* bacteria, showing a change in the shape of the central vein and its partial congestion with blood (A), infiltration of inflammatory cells on its walls (B), hypertrophy in several of hepatocytes (C), and acute necrosis in a others (D), and increase in number and size of Kupffer cells (E). (H&E, X40).

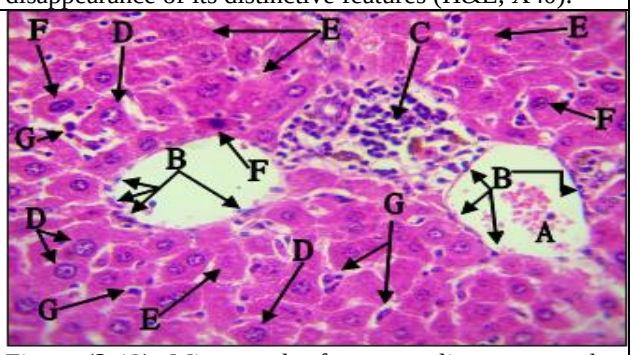

Figure (3-12): Micrograph of a mouse liver two weeks after experimental infection with *S. typhi* bacteria, showing partial blood congestion of one of the two central veins (A), infiltration of inflammatory cells in their walls (B), as well as focal clustering of these cells (C), hypertrophy in several of hepatocytes (D), necrosis of others (E), thickening of the nuclei of some of them (F), and hypertrophy of Kupffer cells (G). (H&E, X40).

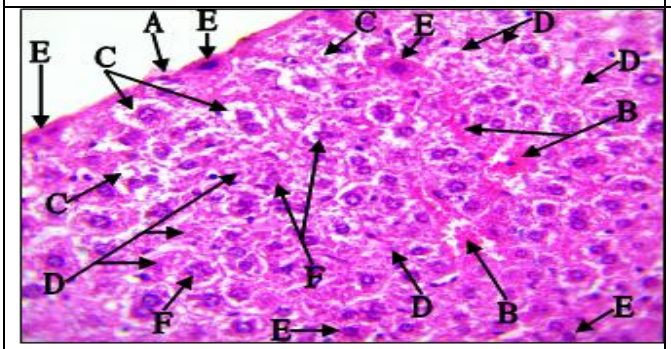

Figure (3-13): Micrograph of a mouse liver three weeks after experimental infection with *S. typhi* bacteria, showing a part of the liver capsule containing inflammatory cells (A), hemorrhage in the tissue parenchyma (B), hypertrophy in several of hepatocytes, as well as the granulated

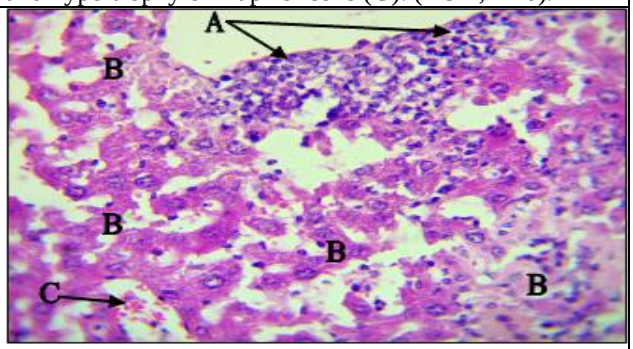

Figure (3-14): Micrograph of a mouse liver three weeks after experimental infection with *S. typhi* bacteria, showing dense focal infiltration of inflammatory cells around the central vein (A), as well as the spread of these cells throughout the tissue section, scattering and lysis of

appearance of the cytoplasm of most of them and its vacuolation (C), necrosis in a number of other cells (D), thickening of a number of nuclei (E), and fragmentation of others (karyorrhexis) (F). (H&E, X40).	hepatocytes (B), huge digitation of the blood sinusoids, and partial congestion of one of them with blood (C), and complete disappearance of tissue features (H&E, X40).

The appearance of somewhat similar histological changes between the *S. typhi* and *S. typhimurium* groups of mice dosed with them provides us with great evidence to understand the pathogenesis of *S. typhi* and its target organs, and that this similarity leads to finding ways that may be sufficient to treat it. One researcher mentioned in a comparative study between *S. typhi* and *S. typhimurium* that there is a great similarity between the histological changes of mice dosed with them [7].

The great evidence obtained through experiments in mice provides valuable information in knowing the degree of infection caused by *S. typhi*, and the number of days it takes to be able to cause infection, as well as providing information about the extent of damage it causes to the liver. However, in contrast, we failed to isolate it from stool, and we were also unable to understand the role of the toxin secreted by *S. typhi* because its expression is restricted to it only and only occurs when it is inside the SCV. A number of researchers reported that they succeeded in causing infection in genetically modified mice that lack the TLR11 structure, and they provided great and important evidence about the role of the flagellum in the pathogenicity of *S. typhi*. However, they failed to understand the immune role of the host, as well as the number of days needed to cause infection, because these mice lack part of the immune system and are therefore more susceptible and sensitive to *S. typhi* [29].

The results of the fourth group, which was treated with the immunosuppressant Cyclosporine for a week, then dosed with *S. typhi* bacteria once, then treated with the Cyclosporine for another week, showed histological changes in it, including acute degeneration of the hepatocytes and sometimes hyperplasia and hypertrophy, and in other sections, noticeable necrosis, which concealed their shape and distinctive radial regularity, with nuclear thickening, thickening of the nuclear membrane and disappearance of chromatin, cytoplasmic vacuolation, focal nodular clustering of inflammatory cells adjacent to the central vein, which appeared congested in several sections, and containing hemolysis in other sections, as well as to an increase in the number and size of Kupffer cells in the blood sinusoids, which varied in their appearance between narrowed, dilated and congested with blood, as in Figures (3-15), (3-16), (3-17), (3-18). (3-19), (3-20).

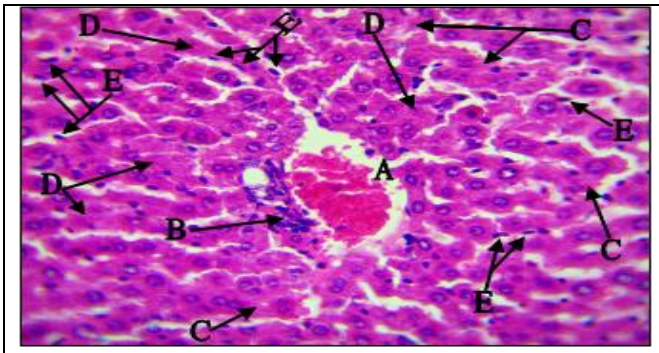

Figure (3-15): Micrograph of a mouse liver after one week of treatment with cyclosporine before and after experimental infection with *S. typhi* bacteria, showing blood congestion in the central vein (A), infiltration of inflammatory cells in one of its sides (B), degeneration in several of hepatocytes (C), and necrosis in another (D), and increase in number of Kupffer cells (E). (H&E, X40).

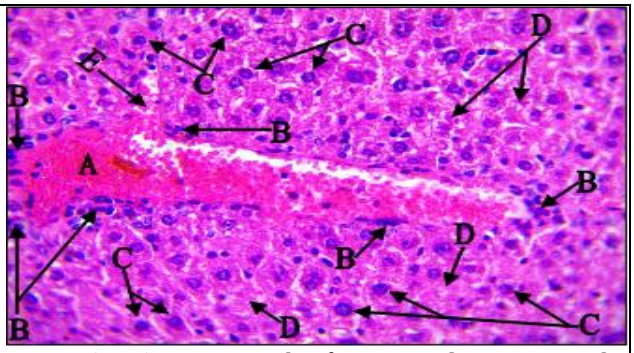

Figure (3-16): Micrograph of a mouse liver one week after treatment with cyclosporine before and after experimental infection with *S. typhi* bacteria, showing acute blood congestion in the central vein and a change in its shape (A), infiltration of inflammatory cells around it (B), thickening of the nuclei (C), necrosis in a number of hepatocytes (D), dilatation of the sinusoids and their congestion with blood (E), acute hyperplasia in all hepatocytes with unclear borders (H&E, X40).

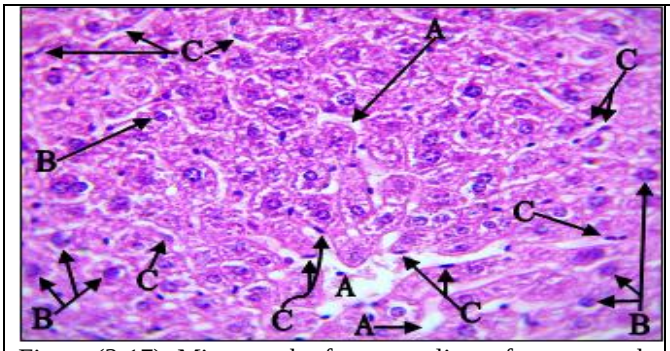

Figure (3-17): Micrograph of a mouse liver after one week of treatment with cyclosporine before and after experimental infection with *S. typhi* bacteria and its dissection after two weeks, showing a part of the central vein partially congested with blood (A), surrounded by inflammatory cells (B), hypertrophy in several hepatocytes (C), degeneration and necrosis others (D), cytoplasmic vacuolation in several hepatocytes (E), thickening of a number of nuclei (F). (H&E, X40).

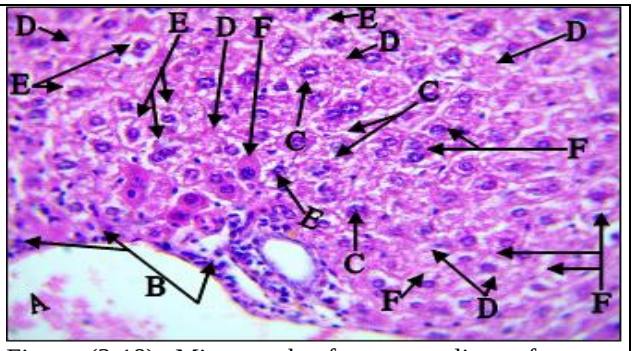

Figure (3-18): Micrograph of a mouse liver after one week of treatment with cyclosporine before and after experimental infection with *S. typhi* bacteria and its dissection after two weeks, showing expansion of blood sinusoids (A), thickening of the nuclei in several hepatocytes (B), an increase in the number and size of Kupffer cells (C), as well as degeneration, necrosis and extensive vacuolation of hepatocytes throughout the tissue section. (H&E, X40).

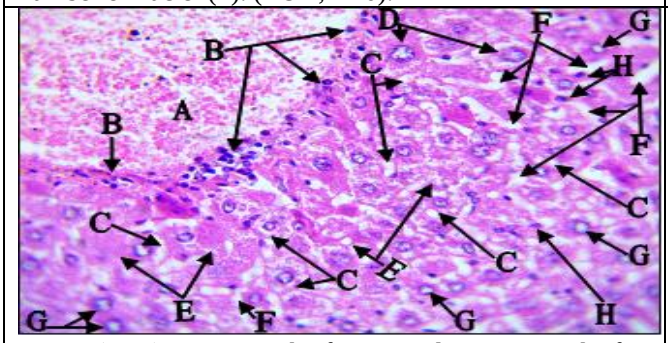

Figure (3-19): Micrograph of a mouse liver one week after treatment with cyclosporine before and after experimental infection with *S. typhi* bacteria and its dissection after three weeks, showing blood congestion in the central vein (A), infiltration of inflammatory cells around it (B), vacuolation of the cytoplasm of hepatocytes (C), hypertrophy of a number of them (D), acute necrosis in others (E), dilation of the blood sinusoids (F), disappearance of chromatin material in a number of nuclei (G), and an increase in the number of Kupffer cells (H). (H&E, X40).

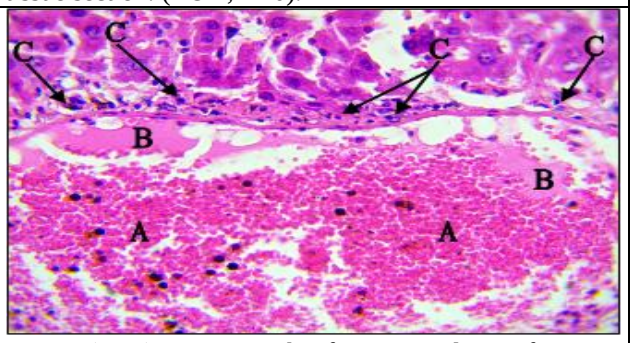

Figure (3-20): Micrograph of a mouse liver after one week of treatment with cyclosporine before and after experimental infection with *S. typhi* bacteria and its dissection after three weeks, showing severe blood congestion in the portal vein (A), partial hemolysis around it (B), infiltration of inflammatory cells around it (C), acute degeneration of hepatocytes throughout the tissue section and their dispersion (H&E, X40).

Cyclosporine is an immunosuppressive drug, a fungal cyclic peptide compound, consisting of 11 amino acids. The mechanism of action of the drug lies in its binding to the cyclophilin protein receptor of lymphocytes, especially T-cells, thus inhibiting the action of the enzyme calcineurin phosphatase, as the enzyme normally activates T-cell receptors and increases intracellular calcium, which leads through calmodulin to activate calcineurin, then calcineurin removes the transcription factor (NF-AT) (Nuclear factor of activated T-cells) that moves to the nucleus of T-cells and increases the transcription of genes to produce the relevant cytokines, especially interleukin-2 (IL-2), which is important for the immune system in its defense against germs [30]. The results of a study indicated that cyclosporine was effective and excellent in suppressing the immunity of mice used in the experiment, which were 6-8 weeks old, when the *Escherichia coli* model was adopted as a cause of sepsis in those mice, and that these mice inhibited by the drug had a mortality rate of about 60% compared to the control group, and the rate of infection in the liver and other organs was high. The study explained that the reason was the decrease in the release of inflammatory substances (cytokines) and the decrease in the production of T-cells in those organs [31]. The results were similar to what other researchers had reached, that the drug was a strong immunosuppressant, and that as well as to its function in inhibition, it was found that it inhibits the production of TLR4 in mice, and also prevents the expression of nucleotide-binding oligomerization domain1 (Nod1), which is an intracellular receptor involved in the innate immune response of phagocytic cells against bacteria, when the Uropathogenic *E. coli* model was used. (UPEC) as a cause of urinary tract infections (UTIs) in mice [32].

We could not find studies on the use of cyclosporine as an immunosuppressant in experimental infection with *S. typhi* in mice, but the existence of studies on its use in the E. coli model is important evidence, as both belong to the Enterobacteriaceae family. A group of researchers reported in their research that cyclosporine, in addition to its functions, increases the adhesion of UPEC bacteria to human umbilical vein endothelial cells, and the most effective concentration was 50 µg/L [33]. The severe liver damage that distinguished it from the other groups can be attributed to the suppression of the immune system by cyclosporine, which allowed *S. typhi* to cause infection, and can be an important indicator in adopting a sensitive animal model for it, as the cause of degeneration and necrosis of hepatocytes can be attributed to the obstruction of mitochondrial functions by toxins. In addition to the toxins common to *Salmonella*, *S. typhi* is characterized by its ability to produce typhoid toxin, as well as the presence of the virulence antigen Vi-Ag in its cell wall composition. These toxins lead to the oxidation process not occurring normally, which leads to the accumulation of fatty acids not linked to ester bonds, which are converted into triglycerides, and degeneration and necrosis occur. Toxins also affect the liver, causing local blood retention in the tissue, which appears as congestion and accumulation of various secretions of the affected cells. The effect is also on the enzymes of the smooth endoplasmic reticulum, which are responsible for removing toxins. They also affect the function of mitochondria in producing the energy needed for the sodium pump responsible for balancing cellular pressure. This is reflected in the appearance of cellular swelling and degeneration. The loss of the nuclear material specific to mitochondria, DNA, is also one of the main causes of cell swelling [34]. A group of researchers were able to infect Wistar rats with *S. typhi*, and the liver was one of the important and targeted organs. Histological sections 5 days after dissection after confirming the infection showed the appearance of blood congestion within the central vein, with infiltration of inflammatory cells into the cavity, degeneration of liver cells, and expansion of dilated sinusoids. They indicated that the reason for this was the toxins of *S. typhi*. In contrast, they stated that rats could overcome *S. typhi* and the liver could return to its normal state [35].

4. CONCLUSIONS

1. The positive IgG/ IgM rapid typhoid test was of low sensitivity in detecting typhoid fever, while a negative result was a good, highly sensitive, reliable indicator of the absence of typhoid fever in people with similar symptoms. As in vitro culture is the best and most accurate method for diagnosing and confirming typhoid fever.
2. Both ALT and AST are a model for detecting liver function in both humans and mice, with ALT being the most important, and both are accepted as a test to confirm typhoid infection.
3. Infection of mice with *S. typhimurium* is the best and most important example in studying the pathogenesis of *S. typhi* in humans.
4. The immunosuppressant cyclosporine is a good choice for making mice susceptible to *S. typhi* infection.

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