Oral Dosing of Male Rats with the Vaccine Prepared from the whole Antigens of Salmonella Typhimurium Loaded on Spherical zinc Oxide Nanoparticles Zn o and Its Histological Effects

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Abstract

Zinc oxide nanoparticles were prepared locally, and tests were carried out using atomic force microscopy to detect the variation in the phenotypic features of these particles, where the shape of the molecular topography appeared with the highest peak up to 18 nm, and the results of the X-ray diffraction test showed the high purity of these minutes, while the microscope test showed the size of these particles averaged 46-59 nm, which is suitable for loading antigens on them, and the shape of the spherical particles was shown. The whole antigen was prepared from local Salmonella typhimurium isolates by local modified global process methods and with superior extraction purity. The results of the lethal half-dose (LD50) test showed that the first dilution 10-8 did not cause the death of any of the rats, and thus it is the LD50 of the whole antigen loaded on zinc oxide nanoparticles. While the phagocytosis index(PI) test showed that this dilution showed the highest index of phagocytosis with an average of 76.24, while the Arthus and late hypersensitivity tests(DTHR) showed that this dilution was the highest reading with an average of 2.694 and 2.872 respectively, and therefore it is the most efficient in the dosing and the occurrence of the immune response, and the immunization began the experimental results for males to rats using a lethal death dose (LD50) showed the emergence of clinical signs that are observed through changes in the animal's appetite, its general condition, the nature of stool and its high temperature. Slowed movement, diarrhea in stool, and a rise in temperature, with an average of 38.5 °C. After giving the booster dose 14 days after the first dose, the pathological changes were less severe and did not show a distinct clinical sign. The results of histological autopsy of (intestines, liver, spleen, kidneys, heart, lung) of rats dosed with whole antigen loaded on nanoparticles of zinc oxide nanoparticles showed slight effects, as their tissues showed some lesions, minor changes and an increase in immune cells, and most parts of their organs were intact, while the members of the control group appeared intact.

Keywords: Salmonella typhimurium, whole antigens, Zn O, male rats and histological effects.

1. Introduction

Salmonella spp. one of the most common human pathogens, Salmonella typhimurium is one of the most prominent types of pathogenic to humans and animals. Ingestion of food and water contaminated with its bacillus, resulting in gastro-intestinal infections, mainly typhoid fever [3][4]. Salmonella spp. have three antigens, the Somatic (O) antigen, which is located within the cell wall of the bacteria and consists of lipopolysaccharides, flagellar (H) antigen It is obtained from bacterial flagella, which are peritrichous flagella. Some types of Salmonella spp. possess a capsular (Vi) antigen that interferes with the somatic antigen [5], it plays a role in the serodetermination of Salmonella spp., and they are called antigens because they it stimulates the immune response and the formation of antibodies when injected into experimental animals [6].

As a result of bacterial resistance to antibiotics, science has turned to nanoparticles as alternatives to antibacterial, secondary particles of metal oxides, whose size ranges from 1-100 nanometers, represent a new trend that is being developed and increasingly given attention for its adoption in research related to medical applications ionic nanoparticles oxides [7]. One of the new effective antibacterial nanoparticles is zinc oxide Zn O, which was registered by the US Food and Drug Administration as a safe material for use [8], as it has many interesting properties such as non-toxicity, low cost, biocompatibility, chemical stability, heat and ability on the absorption of ultraviolet rays, it has made it a promising technique in drug and antigen loading, and food packaging to inhibit microbial growth[9], and it has been used as effective vectors for many drugs intended to target cancer cells[10], as part of biomedical applications due to its biocompatible nature [11], and zinc is an

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essential mineral for the bodies of living organisms, as it is found in many major parts, such as the brain, muscles, bones, etc. [12][13]. It activates many enzymes that help in the synthesis of proteins and DNA [14][15], and it also acts as an antioxidant and helps in the digestion of proteins, blood clotting and bone metabolism [16], and its deficiency leads to the secretion of vitamin a from the liver [17]. This study aimed to prepare zinc oxide nanoparticles Zn O with suitable sizes to load the whole S. typhimurium antigens on them and to reveal their effect on the tissues of inoculated male rats.

2. Materials and Methods

Ethics statement

This research was conducted after approval of the research plan (No. 2344 dated 1/7/2021) by the University of Tikrit, Iraq, and laboratory animals (rats) were obtained from the animal house and the experiment was conducted in it, where all research and ethical standards of the house were adhered to. Animals (1 to 10 of 1990), which comply with the international standards of the Animal Law of 1986, and samples were taken from patients with respiratory infections after approval in kind patients, after taking official approvals from the Salah al-Din Health Directorate, Iraqi Ministry of Health (No. 456, dated 08/14 / 2021), and the instructions for the prevention of diseases and the disposal of research wastes of the Iraqi Ministry of Health.

Experimental animals and experimental protocols

Seventy two male albino rats of the type *Rattus leucopus* were obtained from the animal house at Tikrit University, there were obtained from the animal house of Tikrit University, Iraq, and the experiment was conducted in the animal house under the supervision of animal husbandry specialists, and the rats were left In special breeding cages (each cage contains five rats) at a temperature of 35-37 ° C, lighting for 12 hours a day and a pallet was used to feed them, and I stayed a month before conducting the experiment. And anesthetized before any test in order to reduce pain.

Bacterial specimen collection and identification

10 isolates of *S. typhimurium* were collected from patients with typhoid fever sleeping and attending to Shirqat General Hospital during the period from August 15, 2021, to January 1, 2022, and 5-10 ml of venous blood samples were collected using sterile syringes and placed in special tubes until usage, the samples were cultivated, grown and tested in the laboratories of the College of Basic Education / Al-Sharqat. The samples were planted by streaking method on macConkey agar medium, blood medium agar, XLD medium and SS agar medium, then incubated for 18-24 hours at 37°C. The bacterial isolates were purified according to [18]. The phenotypic and cultivar characteristics of the bacterial isolates were studied according to [19]. The isolates were subjected to microscopic diagnosis according to [20], while the biochemical diagnosis was carried out using oxidase, catalase and IMViC tests according to [21] [22]. Finally, confirmatory diagnosis was performed using Vitek 2 Compact technology.

Preparation of the zinc oxide nanoparticles

The boat was prepared according to what came in [23] and the following steps were followed

1-Dissolve 29.748 g of zinc nitrate Zn $(NO_3)_2$ in 1000 ml of distilled water and add 5 g of starch to the solution and shake until the zinc nitrate is completely dissolved.
2- 100 ml of 1 molar solution of sodium hydroxide Na OH was added to the solution in the form of droplets on the walls of the container and the pH function of the solution was measured until it reached 8.5 and the reaction was stopped.
3-The solution was left for 24 hours, then stagnated and precipitated by centrifugation 10,000 cycles for 5 minutes, and the precipitate was washed with distilled water three times and dried at a temperature of 80 ° C.
4-Burning the product in an electric oven at 400 °C to turn it into a crystalline form and save it after cooling it.

Detection of the properties of zinc oxide nanoparticles

The zinc oxide nanoparticles were examined with an Atomic Force Microscopy (AFM), where the shape and geography of the surfaces were studied, while the results were analyzed according to standard instructions [24][25]. The structural characterization analysis was carried out using X-ray diffraction (XRD) to detect the size of the particles, their crystal structure and the shape of the surfaces, according to the description [26], while the Scanning Electron Microscopy (SEM) was used for the purpose of obtaining microscopic images of zinc oxide nanoparticles while verifying the homogeneity of the examined material. [27]. The phenotypic dimensions of zinc oxide nanoparticles were measured and matched according to [28][29].

Whole antigen preparation

Prepared as mentioned in [30][31] my agencies

1-*S. typhimurium* were grown in Nutrient Agar and incubated at 37°C for 24 hours, they are harvested by physiological salt solution (PBS 7.2), centrifuged refrigerated 3000 rpm at 4 °C for 30 min and then washed three times with PBS, the precipitate is suspended with PBS and placed in the universal tube.
2-Sonication: The universal tube containing *S. typhimurium* bacteria suspended in the Ultrasonicator was placed at 12 cycles with 2 min intervals in between, for 30 min in a cold environment.

3-The acoustic suspension was centrifuged at 10,000 rpm for 30 minutes, the supernatant is then filtered by Millipore filter 45mm.
4-The supernatant was examined by Gram stain and cultured on blood agar to confirm the sterility of this antigen.
5-The total protein concentration of this antigen was measured using Biuret reagent 16 mg/ml and diluted to 0.5 mg/ml.

Whole antigen loading on zinc oxide nanoparticles

The download process was carried out according to the steps mentioned by [32] by my agencies:
1-The zinc oxide nanoparticles were re-dispersed in 25 ml of distilled water at a concentration of 5 mg/ml under continuous ultrasonic sonication to disintegrate the nanoparticles.
2-Loading was performed by incubating different concentrations of the whole antigen (10^{-1},10^{-2},10^{-3},10^{-4},10^{-5}) with zinc oxide nanoparticles at several concentrations (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 mg/ml) under mild agitation at room temperature for 15 minutes to obtain the most efficient loading concentration.
3-The suspension of zinc oxide nanoparticles loaded with antigen was centrifuged at 13200 rpm for 20 minutes, then the amount of antigen in the supernatant was measured with a spectrophotometer at 570 nm.
4-Empty and floating zinc oxide nanoparticles were used to correct the absorbance reading value of the antigen loaded on the zinc oxide nanoparticles and to determine the optical density value.

Determination of the lethal death dose

The lethal half-dose LD50% was determined according to the method of [33], where the whole antigens loaded on spherical zinc oxide nanoparticles were diluted tenfold using physiological salt solution as: (10^{-1},10^{-2},10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8} ,10^{-9}) 36 rats were used, and 4 rats for each dilution, while the control group included rats that were injected with the physiological solution and used 4 rats.

Antigen efficiency test

The immune response in rats to the lethal half-dose of antigens was assessed using two indicators: the phagocytic index (PI) which was prepared according to the steps mentioned by [34], and the Arthus index and delayed hypersensitivity reaction (DTHR), which was prepared according to what he described [35].

Experimental immunization of rats with a lethal half-dose ld50

The method followed by [36] was adopted with some modifications of [37], whereby male rats were divided into three groups, the whole antigen group loaded on zinc oxide nanoparticles, and this group included 6 rats, they were immunized orally with 500 µl of whole antigen loaded on zinc oxide nanoparticles, and after 14 days they are immunized orally with a booster dose for the first dose and the same amount, and after 28 days the challenge dose is given, and left for 3 days before killing and autopsy, and the negative control group includes 6 rats, they are dosed with physiological solution.

Preparation of tissue sections

The rats were anesthetized, then killed, and the following organs were removed from them: (intestines, liver, spleen, kidney, heart and lung) and fixed directly in 10% formalin solution for 24 hours, and tissue pieces were prepared according to what was mentioned]38. [

3. Statistical Analysis

It was conducted using the One-Way ANOVA test (Analysis of variance) with a probability level of p< 0.05 using Minitab Statistical Software]39. [

4. Results and Discussion

Atomic force microscopy was used to detect the surface shape of the zinc oxide nanoparticles used in our study, and the results showed the discrepancy in the phenotypic features of these particles, as can be seen in Fig. 1.

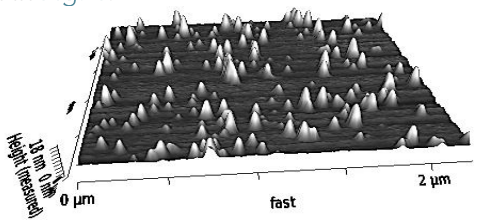

Figure 1. Atomic force microscopy (AFM) detection showing the 3D topography of zinc oxide nanoparticles

While the results of the examination with X-ray diffraction device as shown in Fig. 2, the X-ray diffraction spectrum of nanoparticles corresponds to the main peaks obtained at (100, 002, 101, 102, 110, 103, 200, 112, 201, 004) Bragg reflections at the value of 2 theta for the angles is (31.769, 34.421, 36,252, 47.538, 56.602, 62.862, 66.378, 67.961, 69.033) and 72.560°, respectively. To confirm that the zinc oxide nanoparticles were of high purity, the average size of the zinc oxide nanoparticles was extracted using the Debye Scherer equation, which showed the average particle size from 20-40 nm, and the obtained peaks were compared with the X-ray diffraction database for the global energy of Zn O, which supported this rule with the strong presence of zinc oxide nanoparticles, the results of the current study agree with [40].

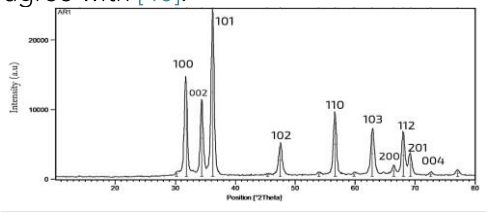

Figure 2. X-ray diffraction (XRD) of nanoparticles of zinc oxide

The results of the detection showed the surface appearance of zinc oxide nanoparticles by means of

a scanning electron microscope (SEM), as in Fig. 3, spherical in shape, and their average size ranged between 46-59 nanometers, meaning that they were within the appropriate nanoscale dimensions and sizes for loading antigens on them.

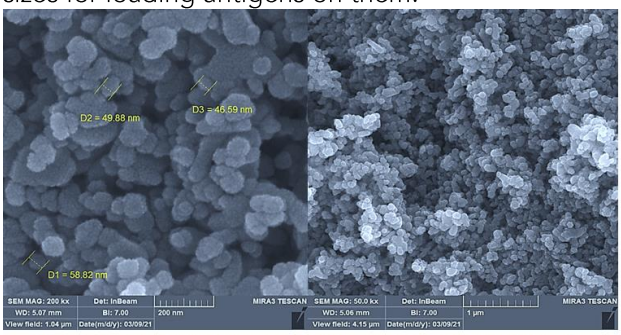

Figure 3. Detection of zinc oxide nanoparticles under an electron microscope (SEM)with two

Figure 3. Detection of zinc oxide nanoparticles under an electron microscope (SEM)with two magnifications of (a):1µm and(b): 200nm.

Several studies have indicated the effectiveness of nanoparticles with diameters between 20-100 nm, as the particles smaller than 20 nm showed secondary toxicity, represented by ineffective cellular filtration and prolonged cellular accumulation]41], it was also found that nanoparticles with a size between 10-10 nm are the most suitable size to be drained into the lymph nodes, and it is also perfectly suitable to be coated with antigens [42][43], and the size, shape, composition and surface properties of Zn O can precisely control nanostructures according to experimental conditions, such as reacting chemicals, solvents, temperature and time [44][45].

The results of whole antigen loading on zinc oxide nanoparticles showed that the absorbance increased when loading the antigen, as it showed an absorbance of 0.741 nm, 50 ml/g was prepared and a standard concentration was counted as 100%, as noted in Table 1.

Table 1. The absorbance of the dilutions used to load the whole antigen on zinc oxide nanoparticles.		
Samples	abbreviation	Absorbance at a wavelength of 570 nm
Zinc oxide nanoparticles	Zn O NPs	0.418
Whole antigen	WA	0.523
Whole antigen loading on zinc oxide nanoparticles	WA + Zn O NPs	0.741

Gomes et al [46] suggested that antigen conjugation of peptides and whole proteins to nanoparticles could lead to their presentation as a multifaceted compound with the ability to cross-link B-cell receptors (BCRs), leading to activation of humoral immunity. Nanoparticles can carry a wide variety of substances from antigens and immunomodulatory to deliver coordinated messages to the immune system that lead to an active immune response [47].

The results of the LD50 test showed that the first 10-8 dilution did not cause death to any of the rats, thus it is the LD50 of the whole antigen loaded on zinc oxide nanoparticles. While the phagocytosis index test showed that this dilution showed the highest index of phagocytosis with an average of 76.24, while the Arthus and delayed hypersensitivity tests (DTHR) showed that this dilution was the highest reading with an average of 2.694 and 2.872, respectively, and therefore it is the most efficient in the dosing and the occurrence of the immune response, as it is noted in Table 2.

xTable 1. The absorbance of the dilutions used to load the whole antigen on zinc oxide nanoparticles.						
Lethal half-dose LD50				Antigen efficiency test		
Dilutions	Death	Revivales	Percentage%	PI	Arthus	DTHR
10-1	4	0	100%	66.2	1.019	1.200
10-2	4	0	100%	68.1	1.150	1.401
10-3	4	0	100%	69	1.350	1.430
10-4	3	1	75%	71.4	1.533	1.584
10-5	2	2	50%	72.9	1.724	1.852
10-6	2	2	50%	73.21	2.112	2.320
10-7	1	3	25%	74.1	2.301	2.550
10-8	0	4	0	76.24	2.694	2.872
10-9	0	4	0			

We have noticed that the rats used in this test were sensitive to the LD50 of 2× 10-8 cells/ml. Chang et al [48] reported that high concentrations of Zn O nanoparticles cause in vivo toxicity and high inflammatory responses and induce lung inflammation by producing inflammatory cytokines from Zn2+ cations and reactive oxygen species (ROS) [49]. The modifier for Zn O has also been used as an adjuvant system to transport various vaccines, and although it has not been fully described, some reports have shown its use as a promising immunomodulatory when combined with antigens [45][50[.

Macrophages are the primary innate defenses of the host against bacterial pathogens attempting to reach the host's body [51], and bacterial antigens activate infections that increase macrophages to reduce their immune-stimulating effect [52], the Arthus reaction is a hypersensitivity reaction type III, which mediates the process of immunocomplex formation with antigen to form immune complexes between antibodies and antigen in the injection site, which leads to activation of the classical pathway of the complement system, and in turn leads to the generation of chemoattractants C3a and C5a that promote neutrophil migration to the injection site. As

a result, an inflammatory response with inflammatory edema forms 3-4 hours after the injection [53][54]. The delayed hypersensitivity reaction represents the fourth type of hypersensitivity reaction and differs from the Arthus reaction in the immune components that participate in it, as it mediates the cell reaction in which specific T-helper lymphocyte (TDTH) plays a major role in association with macrophages and this occurs 24-48 hours after antigen injection, this is the time for TDTH activation by the antigen presented by the phagocytes, and this neutrophil requires production of the cytokines IL-2, IFN-γ and TNF-β that stimulate the migration of more phagocytes to the area of injection, which in turn the enzymes produce extracellular lysozymes responsible for the inflammatory reaction at the injection site [55][56].

Experimental infection began with the appearance of clinical signs that are observed through changes in the animal's appetite, general condition, nature of stool and high temperature. The results of clinical signs were very clear in the infection group given a dose of S. typhimurium antigens a group of rats dosed with whole antigen loaded on zinc nanoparticles by 500 µl and the container at 2 ×10-8 cells/ml. It was noted a deterioration in her health condition, lethargy, slow movement, diarrhea in the stool and an increase in her temperature with an average of 38.5 ° C, while the return to normal was evident in the groups that were treated with the full and whip antigen, and after giving the booster dose 14 days after the first dose, the pathological changes were it was less severe and did not show a distinct clinical sign in the immunized groups, as well as the case in the animals of the control groups. The results of histological anatomy showed that the intestine contained intestinal glands spread in the base plate and lined with columnar cells and other mucous, and those glands were surrounded by white blood cells and macrophages, as for the submucosal layer, it appeared in the form of intermittent colloidal bundles and blood vessels with some leukocytes, and the muscular intestinal wall consisted of a circular inner layer and a longitudinal outer layer, as seen in Fig. 4.

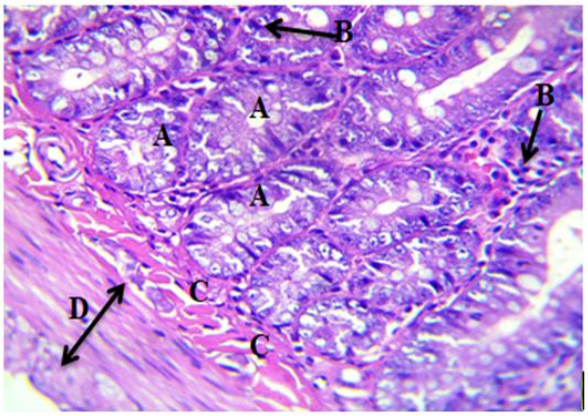

Figure 4. Intestinal tissue Intestinal glands (A) in the base plate and infiltration of leukocytes and macrophages (B) between the intestinal glands, the submucosal layer with colloidal bundles and blood vessels (C) Intestine muscle (D) CH 2 EY 40

Whereas the liver tissue appeared in the form of rows of hepatocytes, and the cells appeared in a polygonal shape with pale spherical nuclei containing one or more nuclei, with the spread of the network of blood granules between the rows of those cells, and it contained a number of kupffer cells and erythrocytes, as seen in Fig. 5(a). As for the central vein in the middle of the liver, blood congestion appeared in it with its continuity at the extremities with the granulocytes containing kupffer cells and the rows of hepatocytes connected to each other radially towards the central vein. Surrounding it are the granulocytes containing kupffer cells and numbers of erythrocytes, and the liver capsule was found thinly from some connective tissue fibers, as seen in Fig. 5(b).

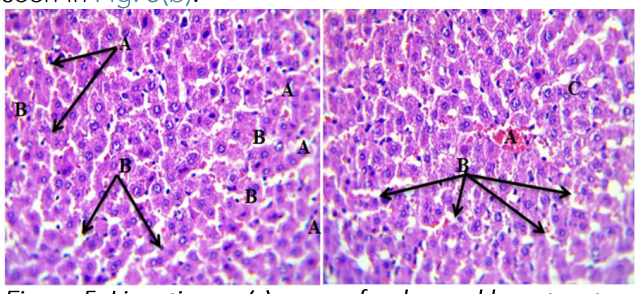

Figure 5. Liver tissue, (a): rows of polygonal hepatocytes (A) faint nuclei, kupffer cells (B) granules 40(C) CH 2 EY 40. (b): Central Vein Hyperemia (A) Granulocyte Network (B) Kupffer Cells, Rows of Radial Hepatocytes Arrangement Around Central Vein (C) CH 2 EY 40

In the spleen, its tissue is surrounded by a capsule of connective tissue containing fibroblasts, and most of the spleen tissue near the capsule has splenic pockets that are almost empty of blood, with the spread of leukocytes and other lymphocytes between the lamellae of the connective tissue and the extension of the spleen tissue in depth accompanied by a dense proliferation of erythrocytes forming the red pulp, as seen in Fig. 6(a). The lymph nodes that make up the white pulp in the spleen are wide composed of lymphocytes and surrounded by the red pulp, and the white pulp center of each lymph node in the spleen tissue contained a nodular artery, which is a small white pulp artery with a thick wall in addition to the presence of numbers of erythrocytes at the vicinity of the white pulp, as noted in Fig. 6(b).

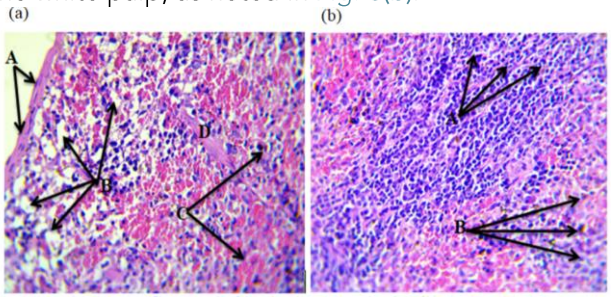

Figure 6. Spleen, (a): Dense fibrous capsule of the spleen (A) splenic pockets free of blood and containing leukocytes (B) erythrocytes (C) red pulp with lymphocytes sulcus fibrous cells (D) CH 2 EY 40. (b): Spleen parenchyma, lymphoid nodules forming white pulp (A) Diffusion of erythrocytes from splenic sinusoids with leukocytes (B) CH 2 EY 40

As for the kidney, its cortex contained renal glomeruli in which atrophy with the eruption of much cytoplasm of epithelial glomerular cells on its surface, in addition to the expansion of the capsular space around the glomeruli and most of the nearby convoluted tubules have epithelial degeneration and loss of some cell nuclei, and the presence of fibrillary edema in the lumen of the tubules with nuclei some cells, as noted in Fig. 7(a). A rupture appeared in the cytoplasm of glomerular cells on the surface of the glomeruli surrounded by the expansion of the capsular space and Bowman's capsule, and the presence of some convoluted tubules appeared intact with the appearance of some inflammatory fibrous filaments in the lumen of a limited number of tubules, and the kidney pulp contained the renal tubules and the thin cusps of loop of Henle are intact with proliferation of leukocytes and macrophages in the interstitial tissue, as seen in Fig. 7(b).

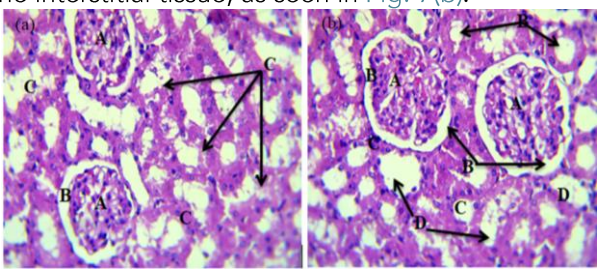

Figure 7. Kidney cortex,(a): glomerular atrophy and the presence of cytoplasmic ruptures of glomerular cells on its surface (A) capsular space expansion (B) Tubular region cell degeneration (C) CH 2 EY40. (b): Ruptures the cytoplasm of glomerular cells (A) capsular space (B)Proximal convoluted tubules (C) Intact distal convoluted tubules (D) CH 2 EY 40.

Cardiac muscle consisted of muscle fibers in the form of bundles parallel to each other with oval-shaped nuclei, and the fascia of muscle fibers consisted of soft connective tissue that contained a number of fibroblasts and leukocytes, as noted in Fig. 8(a). As for cardiac muscle fibers, they were found in the form of a compact mass together, each fiber is surrounded by a loose connective tissue containing scattered fibroblasts and leukocytes, as seen in Fig. 8(b).

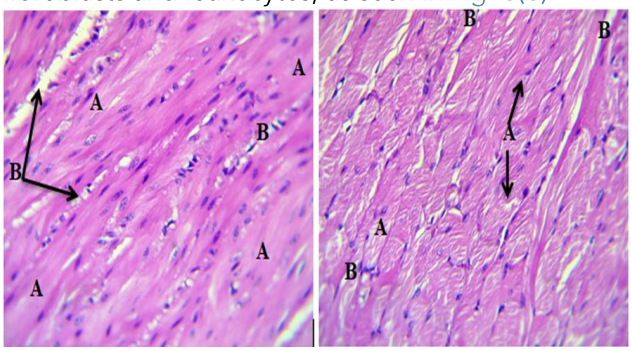

Figure 8. Cardiac muscle, (a): longitudinal direction cardiac muscle fibers (A) fascia of muscle fibers composed of fibrous paving tissue containing leukocytes and fibroblasts (B) CH 2 EY 40. (b): Cardiac muscle fibers strung together (A) fascia muscle fibers (B) CH 2 EY 40.

In the lung, its tissue appeared to be filled with infiltration of inflammatory leukocytes and some alveolar macrophage, with a thickening of the interstitial tissue fibers, and some lung vessels contained limited inflammatory edema, as noted in Fig. 9(a). As for the bronchioles in the lung, there was a breakdown of a number of their epithelial cells lining the bronchioles, and infiltration of leukocytes on the surface of the cells, with an inflammatory infiltration in the form of rows with leukocytes in the lumen of the bronchioles and the edematous infiltrate, and it was also found in the lumen of some alveoli, the blood vessels in the lung thickened with blood and surrounded by numbers of inflammatory leukocytes, as seen in Fig. 9(b).

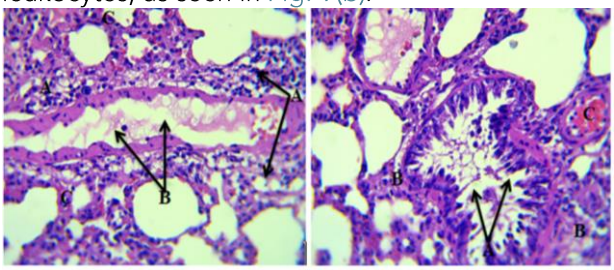

Figure 9. Lung tissue, (a): infiltration of leukocytes (A) with dust macrophages, inflammatory edema (B) in the blood vessels thickening the walls of the alveoli (C) CH 2 EY 40. (b): Destruction and necrosis of epithelial cells (A) in the bronchioles, edematous infiltrate (B) in the alveolar lumen, congestion of blood (C) blood vessels CH 2 EY40.

These infiltrates and outbursts in cells of different organs and the presence of macrophages and leukocytes are only minor effects that can be renewed and repaired, and they are mostly the result of the high immune reaction that occurs in the body of rats as a result of immunization with the whole antigen loaded on zinc oxide nanoparticles that we observed clinically during the experimental immunization that was seen on rats for five days, these lesions can be attributed to what Karmakar et al [57] that Zn O NPs compounds induce systemic toxicity in various organs, especially in the liver, lungs and kidneys. The slight damages that appeared on the organs of male rats in this group may be attributed to what Kuo et al [58] mentioned that the positive zinc nanoparticles lead to pro-inflammatory responses [59][60], as lead to the dissolution of Zn2 + inside cells in lysosomes and the generation of Reactive oxygen species (ROS), which leads to the release of inflammatory cytokines and the cellular activation of immune cells, these effects lead to antigen-specific adaptive immune responses when administered in combination with an antigen [50][61], while Yi et al [62] that nanoparticles with a size between 20-100 nm accumulate in the granular endothelial cells of the liver.

5. Conclusions

Rats dosed with whole antigen loaded on zinc oxide nanoparticles showed a high immune response that lasted for five days, while histological tests showed slight effects on the organs that were histologically studied in the experimental group. Zinc oxide is involved in the loading and transfer of whole antigens and a high cause of safety for these

minutes, so we recommend the side of this study to conduct an immunological study to reveal the nature of the immune response in the dosed rats.

Data availability

The dataset for this study and analysis was created during the research study of the team of authors, is preserved in detail and can be made available once a request is submitted to the corresponding author.

Author contribution

The authors, Ab. Y. S., Ah. Y.S participated in almost all stages of the research except for the conception and design study conducted by B.S.N., A.S.M. and Follow the practical steps for testing by Ab. Y.S., Ah. Y.S, and analysis and interpretation of results, histological anatomy, and interpretation of its results were done by Ab. Y.S., Ah. Y.S, B.S.N., while all the co-authors participated in draft manuscript preparation, Funding for Research and Writing the theoretical part of the research. Finally, the author, correspondent Ah. Y.S, was authorized to communicate with the journal and provide all data related to this research.

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Disclosure statement

There isn't conflict of interest on the part of the authors.

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