

Article

Study of the Therapeutic Effect of Iron Oxide Nanoparticles on Salmonella Enterica Bacteria in the Liver Tissues of Infected Rats

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Abstract: The aim of the study was to demonstrate Fe₂O₃ nanoparticles' antibacterial efficacy against Salmonella enterica. The present study was split into two parts, first in vitro which involved sensitivity test of salmonella enterica against Fe₂O₃NPs. In the second part (in vivo) used 30 albino Rats Mus musculus with weight ranged (160-180) gm. Divided in six groups, (each group consist five rats) as follow: First group: Control group feeding standard diet. Second group: Rats that received an intravenous injection of 1.5 x 10⁸ germs were killed ten days after infection. Third group: Rats that were injected intravenously with 1.5 x 10⁸ bacteria. Then, they were treated for 10 days with 3,2 mg/ml Fe₃O₂NPs (orally) and killed. Forth group: Rats that were intravenously (intra-venously) injected with 1.5 x 10⁸ bacteria. Forth group: Rats injected intravenously (intra-venously) with 1.5 x 10⁸ bacteria. Then treated with 4.2mg/ml Fe₃O₂NPs- orally for 10 days and then killed. Five group: Rats fed with 3.2 mg/ml Fe₂O₃NPs orally for 10 days followed by being sacrificed. Six group: Rats were fed orally with Fe₂O₃NPs at 4.2 mg/ml per day for 10 days and the rats were killed. Many changes were observed in liver tissue of adult mice generally, including infiltration of inflammatory cells in large number forming mass of inflammatory nodules, which are the histological changes. Congestion and hemolysis in must blood vessels. N different stages of necrosis are seen in the nuclei and kupffer cells are present in large numbers in relation to the presence of megakaryocytes and necrosis of the cuboidal cells lining the bile canleculi.

Keywords: Salmonella enterica; iron oxide nanoparticles; Fe₂O₃; antibacterial activity; liver histopathology; infected rats.

Introduction

Salmonella enterica is a major gram-negative bacterial pathogen associated with gastrointestinal and systemic infections [1]. After intestinal invasion, some serovars disseminate to the liver and persist in host cells [2], [3], [4]. Intracellular persistence and antimicrobial resistance have encouraged investigation of alternative antimicrobial approaches. Iron oxide nanoparticles have been studied for their interactions with bacterial membranes and for reactive oxygen species generation [5], [6]. Their activity depends on material properties, concentration and bacterial strain. Shi et al. reported that an iron oxide nanozyme suppressed intracellular S. Enteritidis in cells and infected chickens [7]. Another study found inhibition of S. enterica using fruit-extract-synthesized Fe₂O₃ nanoparticles [8]. These

findings cannot be assumed to apply to the formulation and doses tested here. Iron oxide nanoparticles can also induce oxidative damage in animal cells, making tissue assessment important [9]. Antibacterial findings from copper oxide nanoparticles also illustrate why results from one metal oxide should not be generalized to another [10].

Aims of study

To evaluate the therapeutic effect of iron oxide nanoparticles (Fe_3O_4 NPs) against *Salmonella enterica* infection in the liver tissues of experimentally infected rats.

Materials and Methods

Attenuated steps of *Salmonella Enteritica*

Salmonella Enteritica bacterial strain was standardised for vaccine preparation by the use of spectrophotometer for the preparation of bacterial inoculum. *Salmonella Enteritica* was cultured in media for 24 hrs at 37°C . Following the incubation period, the bacterial culture was diluted with sterile saline solution, so as to obtain a bacterial suspension of OD of 0.5 or 1.5×10^8 CFUs/mL (according to the McFarland standard). This value of optical density was confirmed by optical density reading at 600 nm with a spectrophotometer, in order to have the same amount of bacteria used for the infection induction.

The inoculum was standardized by optical density using the McFarland method.

The Fe_2O_3 NPs were found to be in a highly attenuated state.

Deionized water and a vortex shaker were used to prepare the Fe_2O_3 NPs dilutions. The Fe_2O_3 NPs were added in a test tube containing 1 mL of normal saline to get a concentration of 4.2 mg/mL. In the second test tube 1 mL Fe_2O_3 NPs was mixed with 2 mL normal saline (3.2 mg/mL). The required volumes of each solution were added, and each solution was well mixed by vortex shaking to ensure equal dispersion of the nanoparticles in the solution.

The Fe_2O_3 nanoparticle materials were tested for its antibacterial activity against gram negative bacteria (*Pseudomonas enterica*) isolated from diarrhea patient's by using two concentration (3.2 mg/ml and 4.2 mg/ml) of Fe_2O_3 NPs, and the result is shown as Table (3-3). The antibacterial activity has absent in the Fe_2O_3 NPs against *Pseudomonas aeruginosa*

In Vivo Study

A group of 30 healthy male albino rats of standard weight (160-180 gms) were kept for acclimatization under the environmental conditions of the animal house at The State Company for Drug Industry and Medical Appliances/Samarra for 3 days. The rats were kept in individual cages in a room with a regulated temperature and light/dark cycle of 12 hours. Cleaning of cages was done every day. The rats were randomly allocated into six groups:

Experimental design

In current study (30) albino rats divided to six groups (each group consist five rats) as follow:

1. First group: Control group feeding standard diet, then killed.

2. Second group: Rats injected intravenously with 1.5×10^8 bacteria, and then killed after 10 days from infection.

3. Third group: Rats injected intravenously with 1.5×10^8 bacteria. After that, treated with 3.2 mg/ml Fe_3O_2 NPs- orally for 10 days, and then killed.

4. Forth group: Rats injected intravenously with 1.5×10^8 bacteria. After that, treated with 4.2 mg/ml Fe_3O_2 NPs- orally for 10 days, and then killed.

5- Five group: Rats administrated orally 3.2 mg/ml Fe_2O_3 NPs only for 10 days, and then killed.

6- Six group: Rats administrated orally 4.2 mg/ml Fe_2O_3 NPs only for 10 days, and then killed.

3-3-3- Histological study

Table 1. Antimicrobial activity of Fe_2O_3 NPs against *Salmonella enteritica*

Isolates	3,2 mg/ml of Fe_2O_3 NPs	4.2mg/ml of Fe_2O_3 NPs
<i>Salmonella enteritica</i>	Absent inhibition zone	Absent inhibition zone

Histological sections were prepared using histotechnical method in the Laboratory [11]. The Rats were then anaesthetized by putting them in a sealed glass bottle with a piece of cotton soaked in chloroform, leaving them there for a few minutes until they were not moving and then placed in the dissection basin where they were fixed with pins and the beginning of the abdominal cavity was explained using sharp scissors, scalpels, and forceps, and the desired organs were removed, after which they were washed in the Petri dish containing 0.9% NaCl physiological saline and then put in the fixative solution. After several steps, the sections were stained for 5 minutes in hematoxylin and finally examined by optical microscope.

Result

In vitro study

Inhibition efficacy of Fe₂O₃NPs against salmonella aeruginosa

The antibacterial activity test of Fe₂O₃NPs against *salmonella spp.* at a concentration of 4.2 mg/mL and at 3.2 mg/mL demonstrated no inhibition zone, as shown in figure (1).

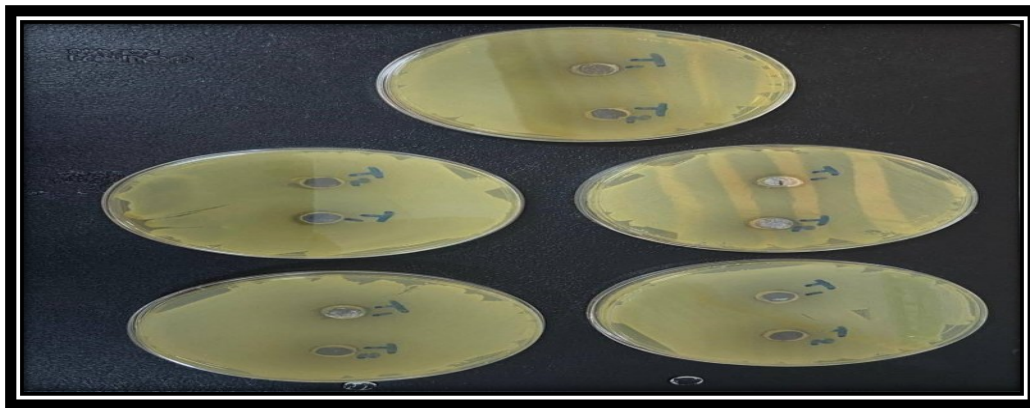


Figure 1. Inhibition efficacy of Fe₂O₃NPs against salmonella aeruginosa

In vivo study

Histological examination

The microscopic analysis revealed that the liver tissue had a normal shape and that the sinusoids, central vein, and hepatocytes were arranged normally.

(Fig: 1)

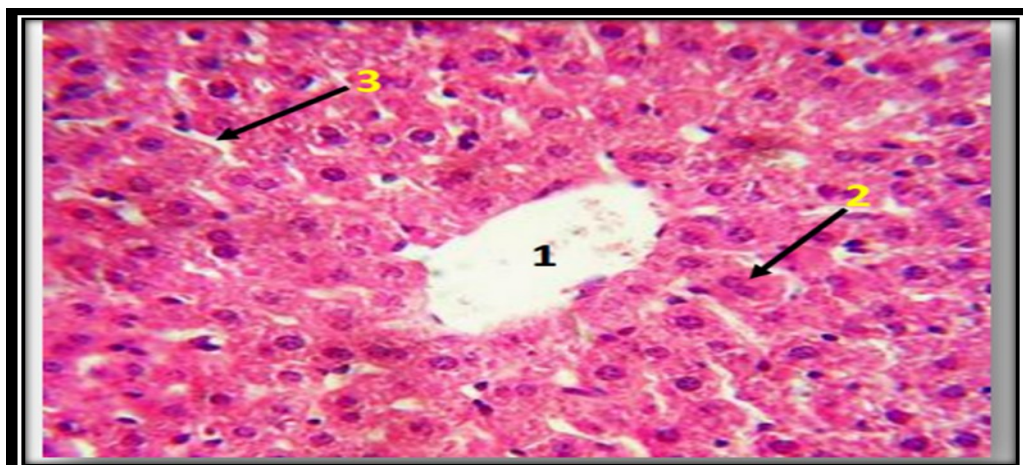


Figure 2. Liver slices from the control group showed normal parenchymal architecture when examined histologically. Radiating cords of distinct, polygonal hepatocytes with conspicuous, central nuclei and uniform eosinophilic cytoplasm encircled the central vein, which looked clear and unobstructed (2). Between the hepatic cords, normal, non-dilated hepatic sinusoids (3) were easily visible, exhibiting no signs of inflammatory cell infiltration or tissue deterioration.(H&E 400X).

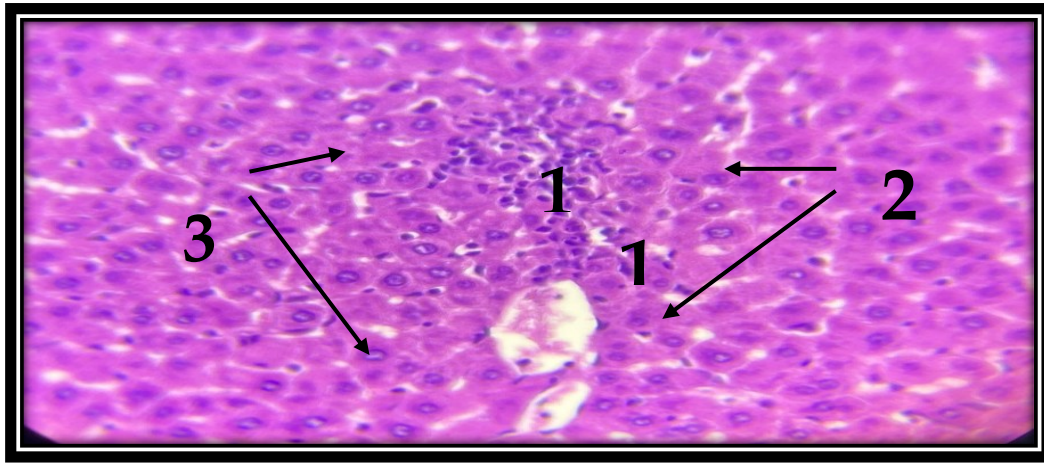


Figure 3. Photomicrograph showing an adult rat's liver segment contaminated with germs. Significant pathogenic changes are shown in 1 (focal inflammatory cell infiltration), 2 (hepatocytes undergoing nuclear karyolysis), and 3 (activated and hyperplastic cells). Kupffer cells that line the sinusoids. (H&E stain, magnification of 400×).

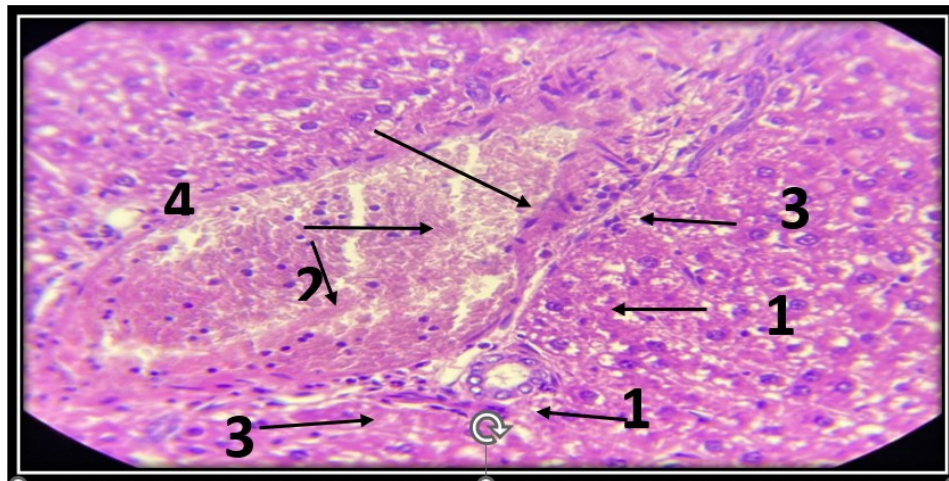


Figure 4. A photomicrograph of a liver histological segment from adult rats given 4.2 mg/ml Fe₂O₃ NPs reveals: Focal inflammatory cell infiltration is shown in 1, hepatocytes undergoing nuclear karyolysis are shown in 2, and activated and hyperplastic cells are highlighted in 3. Kupffer cells that line the sinusoids. (H&E stain, magnification of 400×).

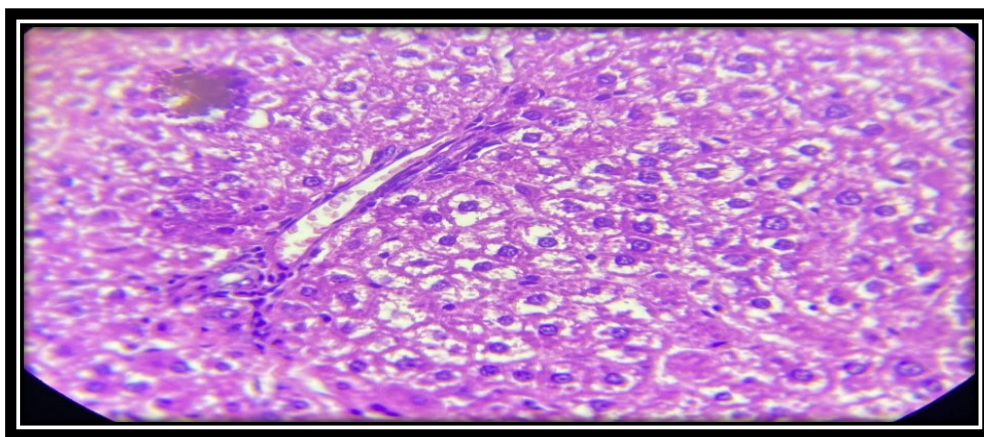


Figure 5. A liver slice from an adult rat given 4.2 mg/ml Fe₂O₃ NPs nanoparticles was photographed under a microscope. Histopathological changes: 1 denotes bile duct epithelial cell necrosis; 2 displays hepatocytes with vacuolar degeneration and altered membrane structure; 3 emphasizes lymphocytic infiltration; and 4 depicts active hyperplastic Kupffer cells. (H&E stain, magnification of 400×).

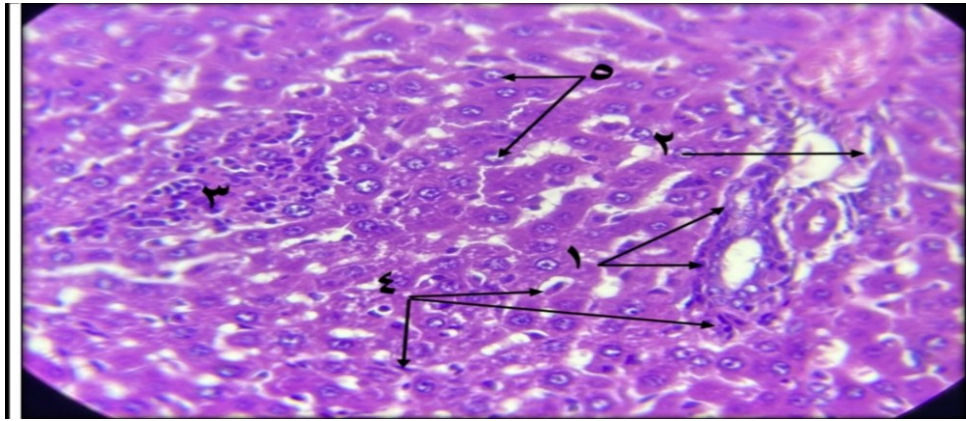


Figure 6. Histological examination of liver sections from adult rats given 3.2 mg/mL Fe₂O₃ NPs nanoparticles (H&E stain, 400× magnification). There are noticeable histopathological alterations in the photomicrograph: Hepatocyte desquamation and degeneration, focal infiltration of inflammatory cells, abundant Kupffer cells, nuclear karyolysis, and necrosis of the cells lining the

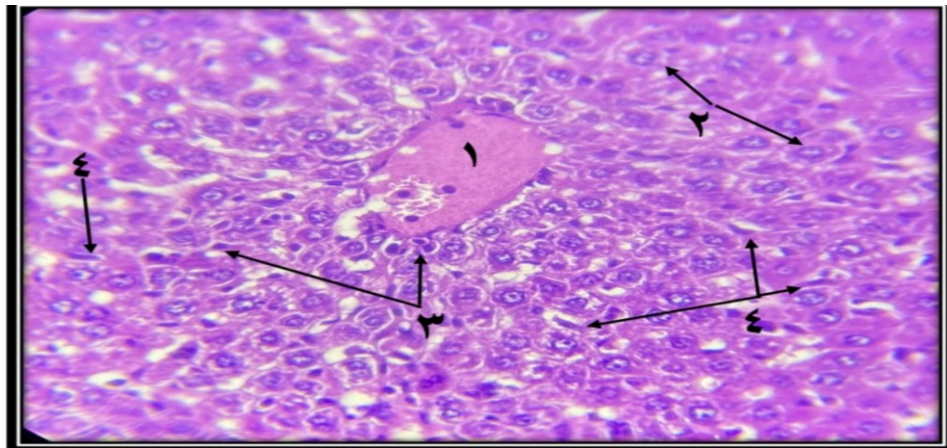


Figure 7. A histological slice of an adult rat's liver treated with about 3.2 mg/ml Fe₂O₃ NPs nanoparticles revealed: (1) intravascular hemolysis; (2) hepatocyte nuclei karyolysis; (3) infiltration of inflammatory cells; and (4) hyperplasia of Kupffer cells. (A&L, 044 almc).

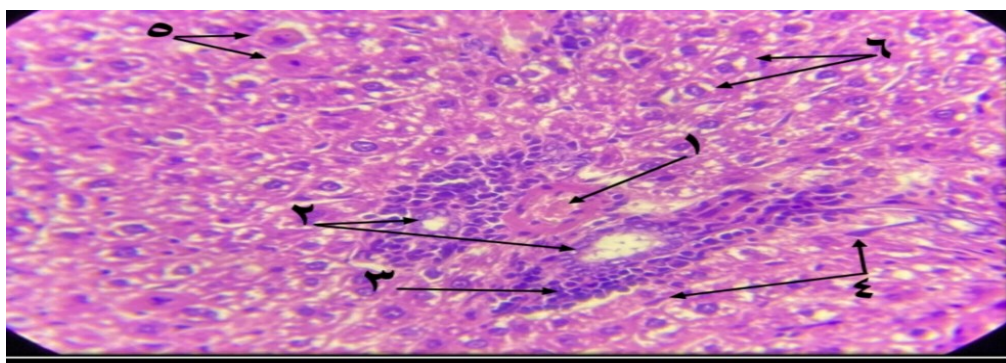


Figure 8. A histological section of the liver from infected adult rats treated with 4.2 mg/ml Fe₂O₃ NPs nanoparticles revealed the following: (1) vascular congestion; (2) hepatocyte nuclei karyolysis; (3) inflammatory cell infiltration; (4) hyperplasia of Kupffer cells; (5) nuclear pyknosis; and (6) thickening of the plasma membrane. (A&L, 044 almc).

Discussion

Under light microscopy, adult mice's liver tissue lost its normal architecture and developed a granular appearance due to inflammatory cell infiltrations in sinusoids. This suggested that the cells that made up the liver tissue had been injured, which resulted in secret chemoattractions that drew inflammatory cells to the infection site in order to eliminate the damaged cells and kill the causative agent (8). According to Riede et al. [9], the only cause of necrosis is cytomembrane damage brought on by ATP deficit, chemical or physical toxins, microbial toxins, and complement factor, which results in permeability disorder. As previously mentioned, chromatin breakage was causing karyolysis to fade out of the nucleus. This is a late indication of programmed cell death, and the toxic effects of medications may alter the membrane permeability of the cells and even the nuclei [10]. As a result, the cells are destroyed, which could result in programmed cell death (Apoptosis). A number of factors can trigger programd cell death, such as the body no longer requires the cells, damage, the need to replace them with new cells, or the end of their vitality [11].

Lamichhane et al., [12] Showed that the liver exhibitions for damage which includes inflammations and necrosis due to poisoning from some drugs and toxic materials, because it was the responsible organ for metabolism, concerning the removal of toxicity by converted into non-toxic material or materials released out.

In addition to presence of hemorrhage and congestion in central vein and sinusoids with hemosedrin granules, Slavin et al., [13] referred to the hemorrhage that is occurred in the tissue due to increased pressure within the blood vessels as a result of the toxic effects of the drug. Subsequently damaged the wall of the blood vessels, and exit the red blood cells. The result was in agreement with our results.

Also distribution of kupffer cells in large numbers with megakaryocytes, necrosis of cuboidal cells lining bile canleculi, Seil et al [14]; AL-Hajj, [15] they showed that the hypertrophy of kupffer cells was caused by determining its activity according to its phagocytic function, as an indicator to enter the foreign bodies. This concept also in agreement with the present study. This study in agreement with [16], [17], [18] when she study the effect of paracetamol (acetaminophen) drug on the liver of pregnancy mice.

Conclusion

Fe₂O₃ nanoparticles showed antibacterial activity against *Salmonella enterica* in vitro. *S. enterica* infection caused marked histopathological damage in the liver, including inflammatory-cell infiltration, congestion, necrosis, and increased Kupffer cells. The tested Fe₂O₃ nanoparticle doses did not clearly prevent these hepatic changes.

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