# Effect of injecting hatching eggs with different concentrations of nano-zinc on the histological characteristics of the liver of Ross 308 broiler chickens

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## Abstract

This study aimed to evaluate the effect of injecting broiler breeder hatching eggs with different levels of nano-zinc on the histological characteristics of broiler liver. The study was conducted through two consecutive experiments. The first experiment took place at the Al-Anwar Company hatchery in Babylon Governorate from October 19, 2024, to November 8, 2024. Seven hundred and fifty fertilized eggs from Ross 308 broiler breeder hens, at 18 days of embryonic age, were used and distributed into five treatments, with 150 eggs per treatment. The treatments included a negative control (no injection)T1 , a positive control (injection of sterile distilled water)T2 , and three treatments injected with concentrations of 14, 16, and 18 ppm nano-zinc at a dose of 0.3 ml/egg within the amniotic sacT3,T4,T5 . In the second experiment, 300 chicks from injected eggs were reared at Al Anwar Poultry Company's farm from November 8, 2024, to December 12, 2024. They were divided into five treatments of 60 birds each, with four replicates (15 birds per replicate.(

The results showed pathological histological changes in the livers of 35-day-old broiler chickens, varying according to the treatment. In the two control treatments, congestion and dilation of the hepatic sinusoids with cellular necrosis were observed. However, the severity of the changes increased in the treatments injected with nano-zinc, including extensive necrosis, liver fibrosis, bile duct dilation, inflammatory cell infiltration, vascular thrombosis, and fatty degeneration, particularly at high concentrations. These results indicate that nano-zinc, especially at high levels, may produce significant adverse effects on the liver tissue of broiler chickens.

Keywords: nano-zinc the histological characteristics , injecting hatching eggs.

## Introduction

In-ovi injection/feeding has gained significant attention in the poultry industry because it offers an effective way to improve the embryo's physiological utilization of nutrients during the final and critical stages of embryonic development. By delivering minerals, vitamins, and nutrients directly to the embryo within the egg, it supports the maturation of the digestive system and promotes increased nutrient absorption after hatching. This early support often results in higher growth rates, improved feed conversion efficiency, enhanced immune system function, support for skeletal development, and reduced early chick mortality rates after hatching [1, 2]. Nanoparticles can be considered a modern approach to early feeding, as they enable more effective delivery of nutrients to the embryo during its developmental stages, opening new horizons in embryonic nutrition and poultry performance improvement techniques. Recent studies have shown that in-ovi injection using metal nanoparticles such as zinc and copper can be a promising technique in embryonic nutrition and explore the potential of nanotechnology in animal nutrition and production [2]. Nano-zinc was specifically chosen due to its vital role in stimulating cell proliferation and promoting growth, as well as its contribution to improving fertility, enhancing immune system function, and regulating gene expression. Zinc is also an essential element in the activity of many vital enzymes and is directly involved in various metabolic pathways within the body [3]. Furthermore, zinc plays a crucial role in cellular defense against oxidative stress as a key component of the enzyme superoxide dismutase, in addition to its active contribution to the development and functional efficiency of the immune system in broiler chickens [4]. The liver is an accessory gland of the digestive system, playing a vital role in the production of bile and contributing to the synthesis of many proteins, enzymes, and chemical compounds necessary for digestion [5]. The liver is also characterized by its ability to produce a range of antioxidant enzymes that neutralize free radicals and hydrogen peroxides, such as catalase and glutathione peroxidase, thus contributing to the protection of cells from oxidative damage [6]. Furthermore, the liver plays a crucial role in detoxifying the body through a series of chemical reactions aimed at neutralizing and binding toxic substances, which are then excreted with bile for later elimination in feces [7]. Given this vital function of the liver, the importance of nano-zinc as a promising new technology in the poultry industry becomes clear.. Accordingly, the present study was designed to assess the impact of in ovo injection of varying concentrations of nano-zinc on the histological characteristics of the livers of Ross 308 broiler chickens.

## Material and Method

Both experiment were conducted at Al-Anwar Poultry Company in Babylon Province : the first experiment was involved eggs injection in the hatchery from October 19, 2024, to November 8, 2024 whil The second experiment included raising the chicks at the farm, from November 8, 2024, to December 12, 2024

### .1First Experiment:

### .1-1Source of Experimental Eggs

The eggs were obtained from the Al-Anwar Poultry Company hatchery (imported from the Turkish company BEYZA). 1200 hatching eggs were individually weighed using an SF-400 Electronic Kitchen Scale (broiler breeder hens, type ROSS 308), with an average weight of 61 g ± 1 g. Dirty or deformed eggs were discarded.

### .2-1Egg Incubation

On October 19, 2024, were weighed and placed in the incubators. Petersim incubators, manufactured in Belgium, were used. The incubator temperature was maintained at 37.8°C and the humidity at 85-88%. Egg turning was automatic.

### .3-1Solutions Used for Egg Injection

Sterile distilled water was used to dilute and prepare the zinc nanoparticle solution. The zinc nanoparticles were obtained from Nanosany Corporation in Iran, with a particle size of 10-30 nm and a purity of 99%. The solutions were then prepared as follows:

(1Mix 1 ml of zinc nanoparticles with 1000 ml of sterile distilled water to obtain a concentration of 1000 ppm.

(2Take 1.4 ml of the zinc nanoparticle solution and dilute it in 100 ml of sterile distilled water to obtain a concentration of 14 ppm.

(3Take 1.6 ml of the zinc nanoparticle solution and dilute it in 100 ml of sterile distilled water to obtain a concentration of 16 ppm.

(4Take 1.8 ml of the zinc nanoparticle solution and dilute it in 100 ml of sterile distilled water to obtain a concentration of 18 ppm.

4-1Method of Injecting Hatching Eggs

On 5/11/2024, at 18 days of incubation, the eggs were injected into the amniotic fluid according to the following steps:

•Prepare the injection solution according to the specified concentrations.

•Before starting the injection, place the solution in the injection chamber to ensure the solution reaches a temperature of approximately 30°C to prevent shock to the embryo.

•Eggs were examined by candling. Unfertilized eggs or those with dead embryos were excluded. 750 eggs were selected from 1200 eggs and divided into five treatments of 150 eggs each. The injection procedure was performed by sterilizing the injection site with alcohol-soaked cotton and the drill. The eggshell was then pierced with the drill, taking care not to crack the shell or damage the embryo's blood vessels. A nano-zinc solution was then injected using an automated syringe calibrated to 0.3 ml into the amniotic sac. After injection, the puncture was sealed with paraffin wax, and the eggs were placed in the incubator after being labeled until hatching.

5-1Egg Injection Treatments

(1Treatment T1: No injection (negative control.(

(2Treatment T2: Injection with sterile distilled water (0.3 ml/egg) (positive control .(

(3The third treatment (T3 ) involved injecting 0.3 ml/egg of a nano-zinc solution at a concentration of 14 ppm.

(4The fourth treatment(T4) involved injecting 0.3 ml/egg of a nano-zinc solution at a concentration of 16 ppm.

(5The fifth treatment (T5) involved injecting 0.3 ml/egg of a nano-zinc solution at a concentration of 18 ppm.

.2The second experiment (the field part of the experiment): On November 8, 2024, the newly hatched chicks were transferred from the hatchery to the farm. The chicks were raised from November 8, 2024, to December 12, 2024, after which the laboratory analyses for the study were conducted.

Chick Management

After the chicks arrived at the rearing hall, 300 chicks were taken from the total number of chicks hatched from 750 eggs (60 chicks per treatment) from the total number of chicks hatched from 150 eggs per treatment. The chicks were distributed among 5 treatments with 4 replicates 15 chicks They were randomly distributed within pens, each pen measuring 1 x 1.5 m. The chicks were fed a starter diet (23.04% protein and 3021.45 kcal/kg feed) from day one until the third week of age. This was then replaced with a grower diet (20.06% protein and 3194.92 kcal/kg feed) until the end of the fifth week. Feed and water were provided ad libitum. The diet used is shown in Table 1:

## Histological Characteristics

### .1Preparation of Histological Sections:

Two birds from each replicate (eight birds per treatment) were slaughtered at 35 days of age. Histological samples were taken as quickly as possible to ensure no damage to the bird's body tissues. Two centimeters of liver were cut, and all sections were washed with tap water and placed in tubes containing 10% formalin. The procedures were performed according to [9.[

### .2Examination of Histological Sections

The histological sections were examined using an Olympus BH2 compound microscope (of Japanese origin) and photographed using a digital camera connected to a Lenovo computer.

## Results and Discussion

### Histological Characteristics of the Liver at 35 Days of Age

Histological sections of the livers of the experimental birds at 35 days of age were studied (Figures 1–10). The results of the control treatment (without injection), shown in Figures 1 and 2, revealed mild to moderate congestion and sinus accumulation, accompanied by partial loss of normal liver tissue structure. Figure 2 of the same treatment also showed marked congestion and significant dilation of the inlet area and hepatic sinuses, in addition to hepatocyte necrosis. In the T2 second treatment (injection with distilled water), Figures 3 and 4 showed severe hepatocyte necrosis with cavitation in the liver tissue and red blood cell infiltration, as well as marked congestion of the central vein.

The histological sections of the third T3 treatment, shown in Figures 5 and 6, demonstrated extensive hepatocyte necrosis accompanied by severe lobular fibrosis, along with bile duct dilation and significant inflammatory cell infiltration.

Figures 7 and 8 from the forth T4 treatment group showed extensive liver fibrosis with significant proliferation of fibrous tissue, as well as vascular thrombosis and central venous thrombosis.

Histological sections of the livers from the fifth T5 treatment birds, shown in Figures 9 and 10, revealed pronounced fatty degeneration accompanied by significant dilation of the central venous system, in addition to thrombosis and epithelial hyperplasia. The liver in poultry is the primary organ responsible for detoxifying and purifying the body of toxins, whether they originate from feed components or from internal metabolic byproducts such as free radical formation. Zinc nanoparticles have a high affinity for generating reactive oxygen species (ROS) within liver cells as a result of their reaction with oxygen in the cellular environment. This leads to attacks on cell membranes, proteins, and DNA, causing direct cellular damage. This damage disrupts antioxidant defense systems, particularly glutathione and superoxide dismutase, leading to liver cell death (necrosis), liver tissue damage, and cell membrane breakdown, as well as an increased inflammatory response due to the activation of hepatocytes [10]. When nanoparticles are distributed throughout the body, they tend to accumulate in the liver, a specialized detoxification organ, leading to partial blockage of capillaries and redness of the tissues. The accumulation of these particles also stimulates the release of inflammatory mediators, contributing to increased clustering of inflammatory cells around blood vessels. This leads to telangiectasia (dilated capillaries), slowed blood flow within the liver tissue, and fluid accumulation—a condition known as hepatic congestion [11.[

Furthermore, damage to the cell membrane and loss of intracellular energy can cause cell rupture and the tearing of its contents, a process known as necrosis. This often occurs with the use of high doses of nano-zinc, particularly through injection at high concentrations or direct exposure to liver cells [12.[

***Table 1: Feed Ingredients and nutrient composition of the experimental diet***

| Diet ingredients | Starter % | Finisher% | ) |
|---|---|---|---|
| Corn | 30.10 | 40.00 | |
| Wheat | 28.16 | 24.00 | |
| SBM (48% CP) | 31.74 | 24.80 | |
| Protein concentrate* | 5.00 | 5.00 | |
| Veg oil | 2.90 | 4.40 | |
| Limestone | 0.90 | 0.60 | |
| Dicalcium phosphate (DCP) | 0.70 | 0.90 | |
| Vitamins and Minerals Premix | 0.20 | 0.20 | |
| Nacl | 0.30 | 0.10 | |
| Calculated % | | | |
| ME (kcal/kg) | 3021 | 3195 | |
| CP | 23.04 | 20.06 | |
| Lysine | 1.27 | 1.07 | |
| Methionine | 0.41 | 0.38 | |
| Cysteine | 0.35 | 0.30 | |
| Methionine + cysteine | 0.82 | 0.78 | |
| Available phosphorus | 0.41 | 0.43 | |
| Energy: | 131.14 | 159.77 | |

*

Protein concentrate type W Special 5 – Brocon: Made in China Each kg contains 40% crude protein, 5.3% fat , 1% fiber , 6% calcium , 3% available phosphorus , 25.3% lysine , 90.3% methionine+cysteine , 2.2% sodium , 2100 kcal / kg metabolized energy , 20000 IU vitamin A , 40000 IU vitamin B3 20 mg, 150 mg vitamin B2 + B1 mg , 15 mgMeans within a column, row and their interactions followed with the same vitamin K3, 30 mg vitamin E, 500 mg vitamin D3, 300 mg vitamin B6, 300 mg vitamin B12, 10 mg folic acid , 100 microgram biotin, 1 mg iron , 100 mg Copper, 2.1 mg Manganese, 800 mg Zinc , 15 mg Iodine, 2 mg Se, 6 mg Cobalt , 900 mg Antioxidant ( BHT.(

** Chemical analysis of the ration was calculated according to the NRC . 1994. [8[

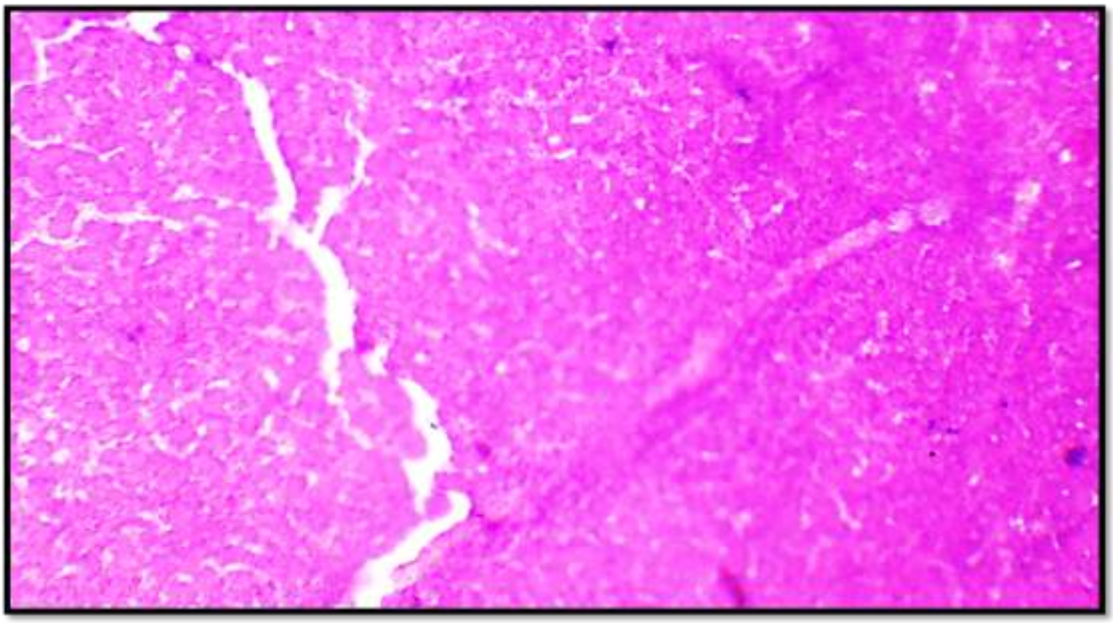

Figure (1) Photomicrograph of liver of T1 group, showed mild to moderate sinusoidal congestion and dilatation (black arrow) , loss of normal architect ( yellow arrow ) ( H & E 10 X(

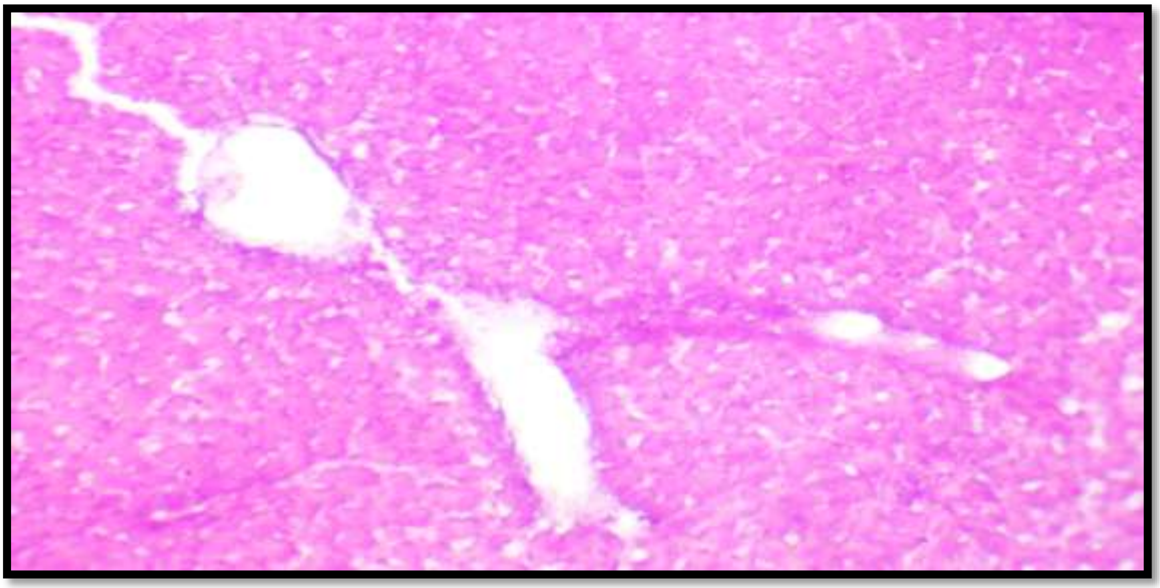

Figure (2) Photomicrograph of liver of T1 group, revealed significant congestion and dilatation in the portal area and sinusoides (black arrow), necrosis of hepatocytes( yellow arrow) . (H and E, 10X .(

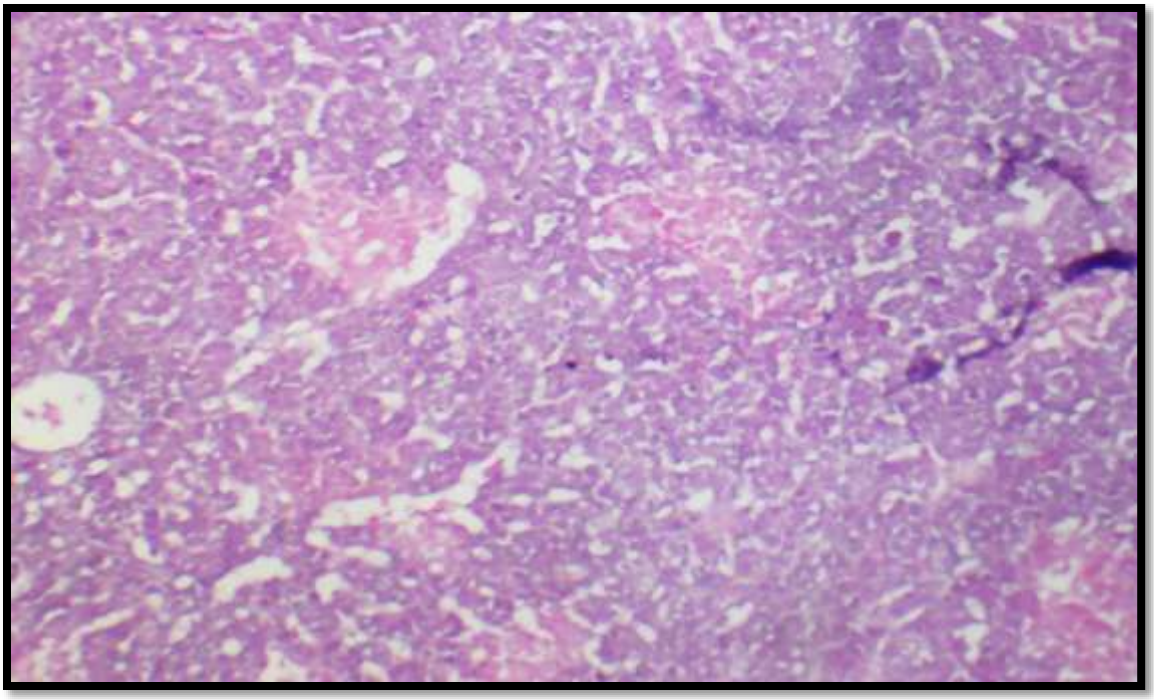

Figure (3 ) Photomicrograph of liver of T2 group, revealed significant hepatocellular necrosis and vacuolation (black arrow), RBCs cellular extravasation

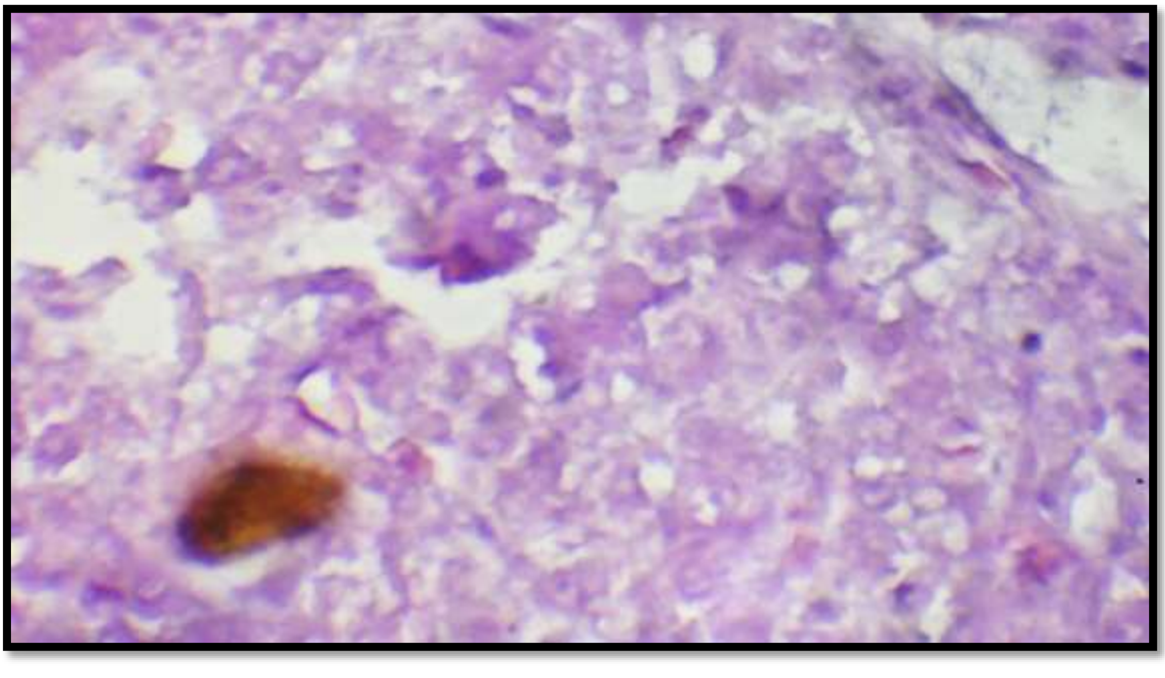

Figure (4 ) Photomicrograph of liver of T2 group, revealed significant hepatocellular necrosis and vacuolation (black arrow), RBCs cellular extravasation ( yellow arrow) and congestion of central vein(red arrow) . (H and E, 10 X (

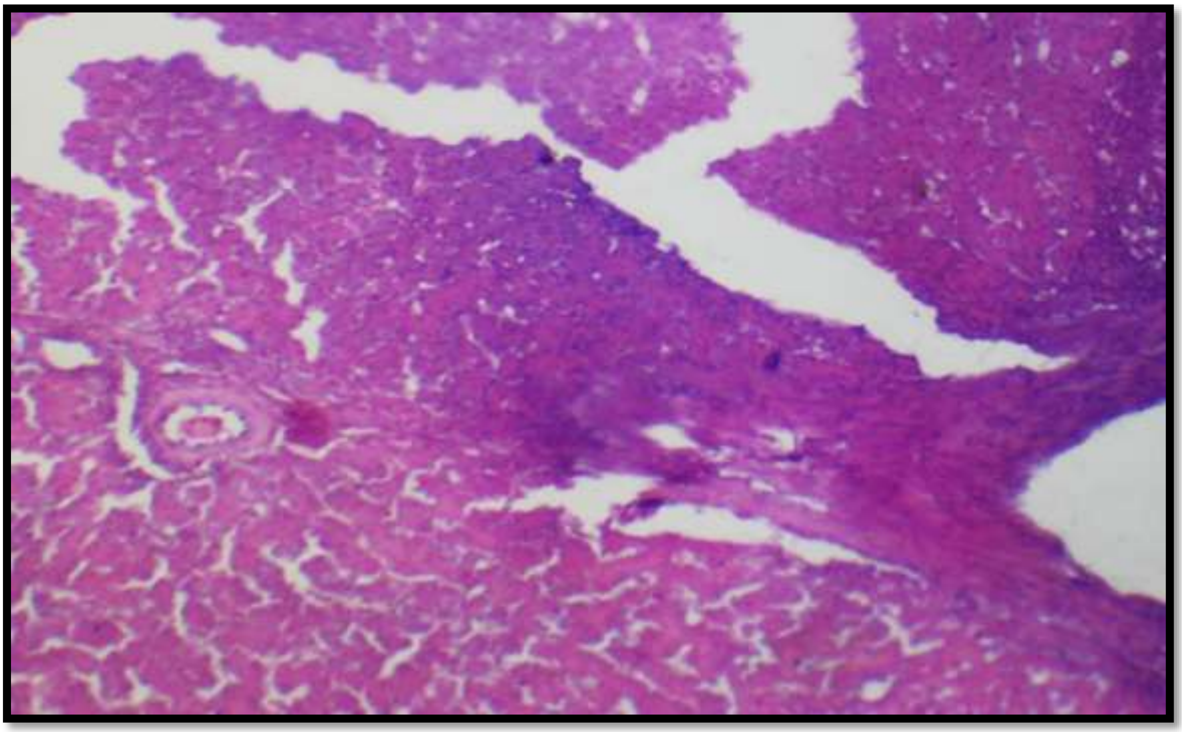

Figure (5 ) Photomicrograph of liver of T3 group, showed extensive necrosis (black arrow), sever lobular fibrosis ( yellow arrow) with significant bile duct epithelial

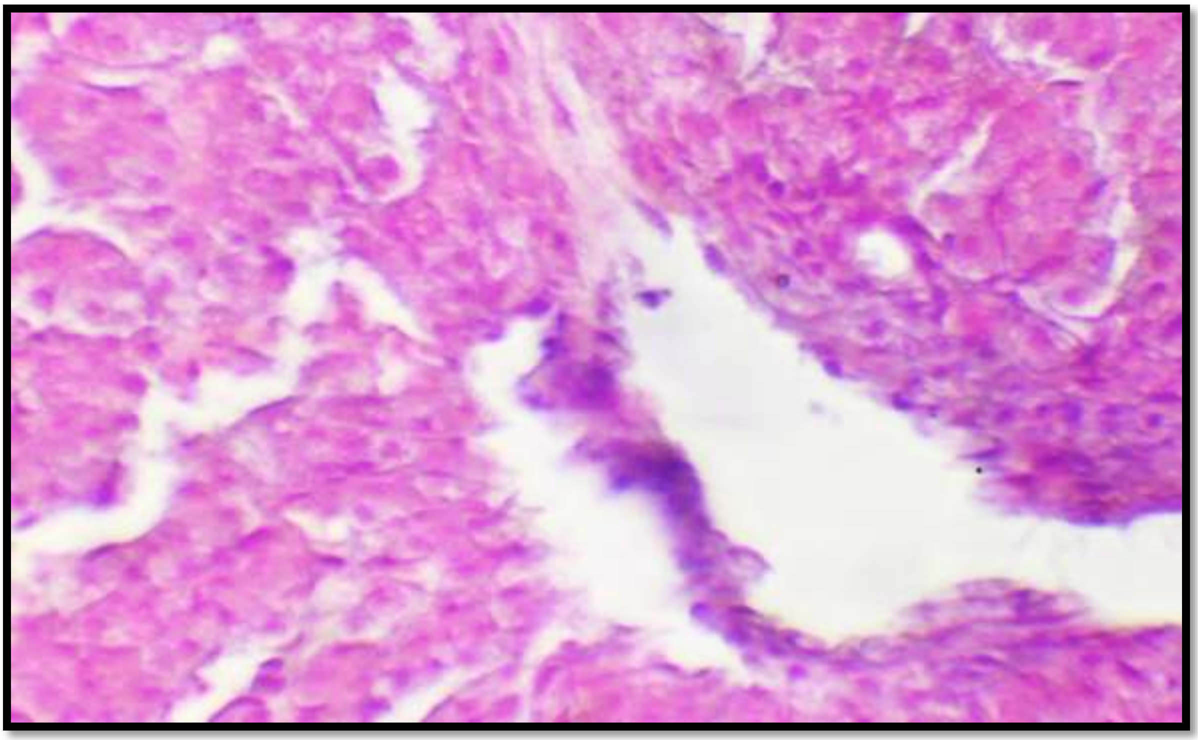

Figure (6 ) Photomicrograph of liver of T3 group, showed sever perivascular inflammatory cells infiltration (black arrow), sever hepatic necrosis ( yellow arrow) with hepatic depletion (red arrow) . (H and E, 40X .(

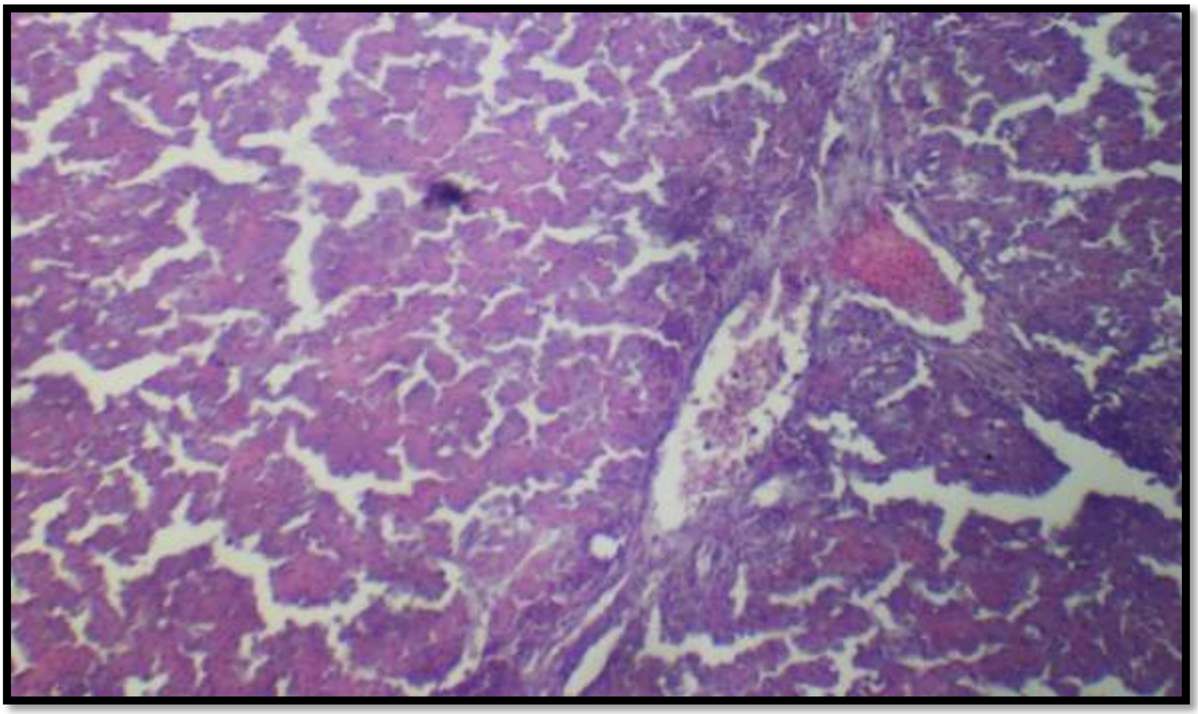

**Figure (7) Photomicrograph of liver of T4 group, showed extensive hepatic fibrosis (black arrow), sever periportal fibrous tissue proliferation ( yellow arrow) with**

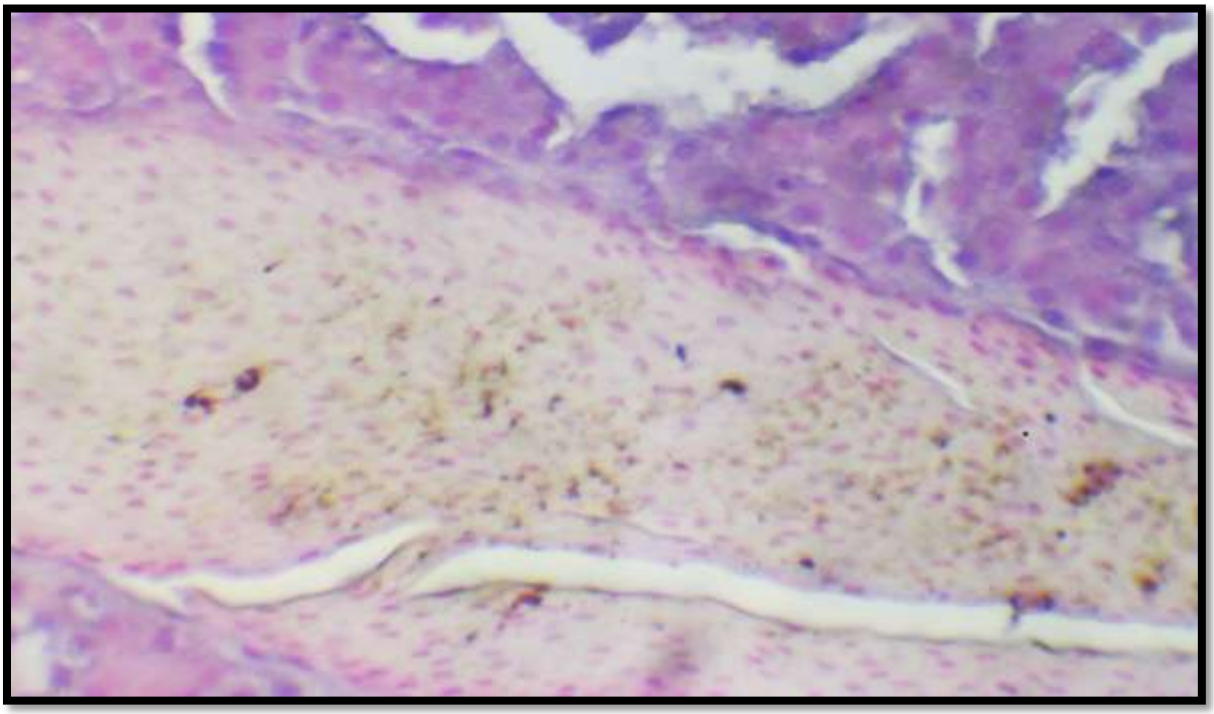

Figure (8 ) Photomicrograph of liver of T4 group , showed massive thrombus inside central vein (black arrow), sever hemosiderosis ( yellow arrow) with MNCs proliferation (red arrow). (H and E, 40X .(

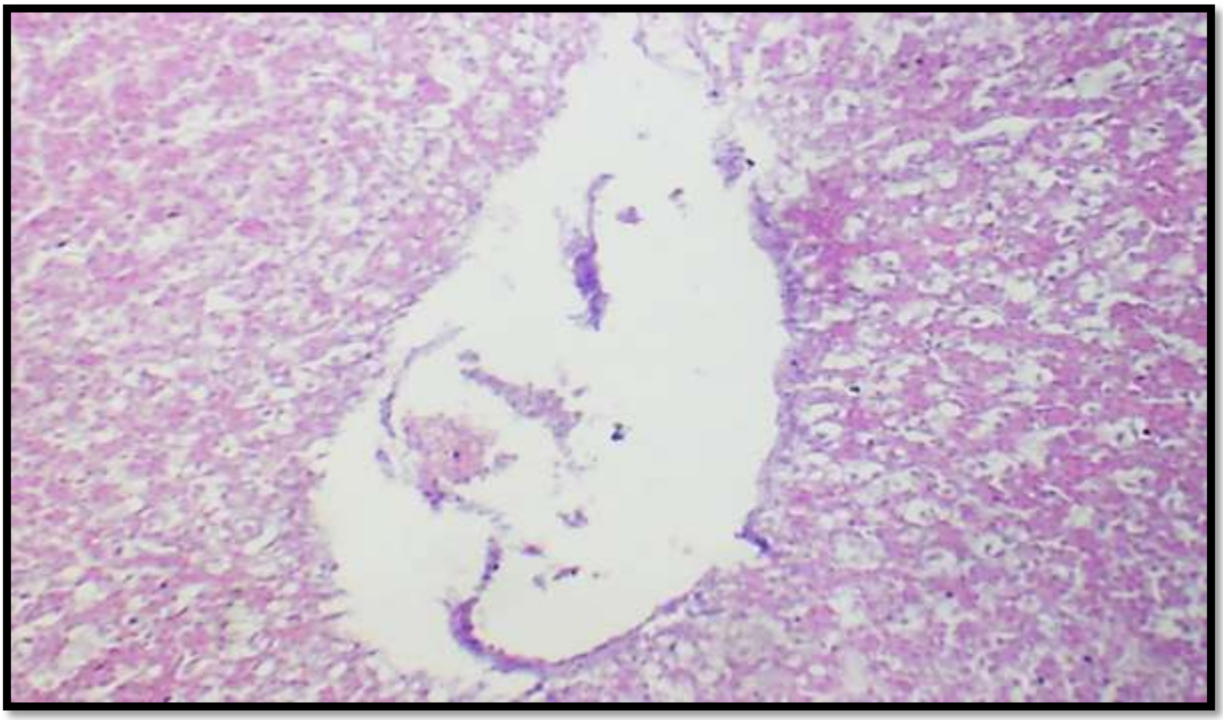

Figure (9 ) Photomicrograph of liver of T5 group , , showed sever vacuolation (fatty degeneration) (black arrow), sever central vein dilatation ( yellow arrow) with MNCs proliferation (red arrow). (H and E, 10X(

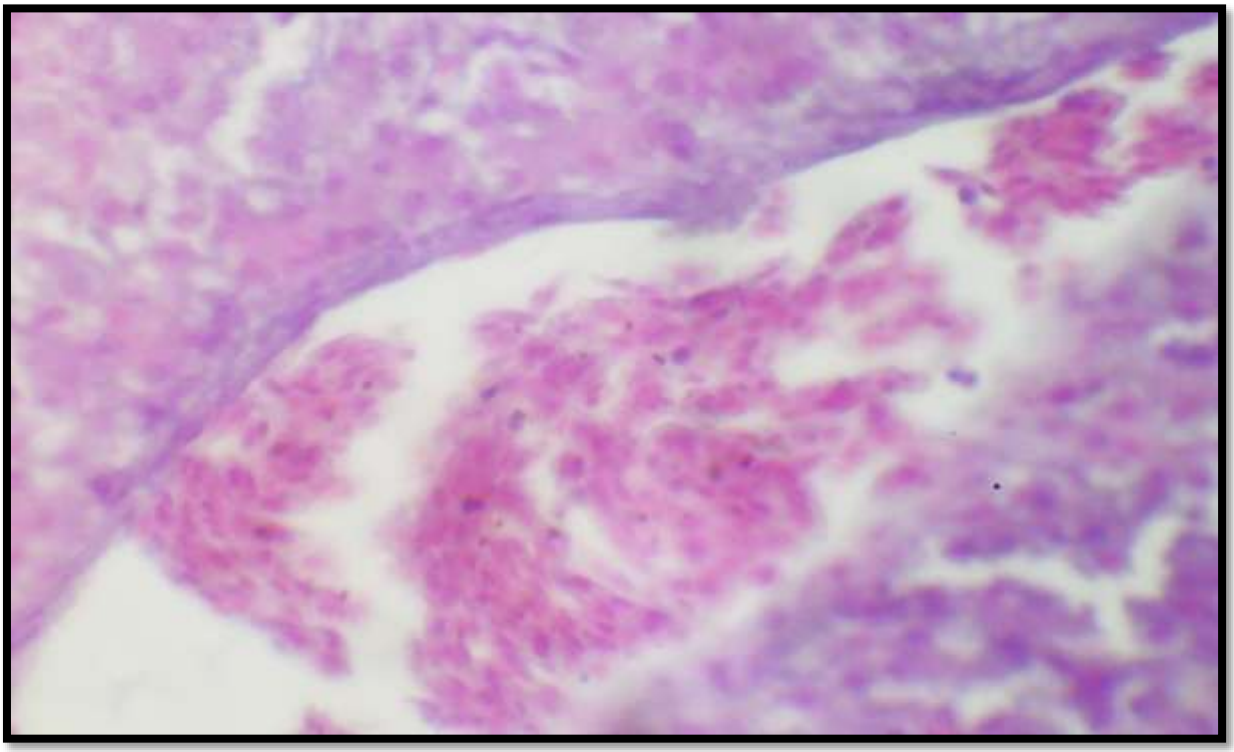

Figure (10 ) Photomicrograph of liver of T5 group , showed sever thrombosis (black arrow), sever vascular epithelial hyperplasia ( yellow arrow). (H and E, 40X .(

## Conclusion…

Our study revealed that injecting hatching eggs with nano-zinc directly affects the histological structure of broiler livers, with the severity of pathological histological changes increasing with the concentration of nano-zinc used. High concentrations were found to cause significant liver damage, including necrosis, fibrosis, fatty degeneration, and inflammatory cell infiltration, compared to control treatments. Therefore, it can be concluded that using nano-zinc in hatching eggs, particularly at high levels, may have adverse effects on the liver health of broilers, necessitating caution in determining its concentrations and seeking safe levels for use.

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