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Toxicological effect of zinc on liver of broiler chicks

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Abstract

Background: The objective of this study is to examine the toxic effects of zinc on the liver of broiler chicks. For this purpose, twenty broiler chicks were taken for the experiment and their weight ranging from 35-45 g. They were divided into four groups: one control and three treated groups. All treated groups were supplemented with 300 mg/kgb.w (low dose, LD), 600 mg/kgb.w (intermediate dose, ID) and 900 mg/kgb.w. (high dose, HD) of Zinc and the control group was fed basal commercial starter diet for 21 days.

Results: Necrosis, liver cell hypertrophy, fuzzy liver cells and lymphocytic inflammation were found in birds exposed to the low and intermediate dose as compared to the controlled group. Broiler chicks exposed to high dose showed pronounced changes in the liver such as congestion of blood vessels, connective tissue hyperplasia, bile duct proliferation, dilation of sinusoids, damaged intercellular contacts between hepatocytes, liver cell hypertrophy and accumulation of inflammatory cells

Conclusion: After the careful analysis of the study, the results have been reported that if one can take zinc in higher amount as supplements or in their foods than it affects the normal structure of the liver and it will alter the functioning of the liver in both human and animals.

Keywords: Zinc, Liver, Broiler chicks

Background

Zinc is a naturally occurring trace element and it is ubiquitous in the environment. The average concentration of zinc in the earth's crust is about 70 mg/kg. Zinc is not available in the environment in its free form, but it is available with the various elements such as zinc carbonate, zinc oxide, zinc sulphide and zinc chloride. The mining activities and metal smelters are primary sources of zinc [3, 23]. Even it can be produced in the pure form by the electrolytic process. The normal concentration of zinc in plants and vegetables is about 15 to 100 mg/kg because zinc ions are strongly adsorbed to the soil which is taken up by the plants. Zinc compounds are used as catalysts, fertilizers, batteries, photographic paper, textiles, medical, household applications, cosmetics, paints, plastics and as nutritional supplements [4, 10, 13].

Zinc is an essential nutritionally indispensable element for human, animals and plants because it plays an important role in various biological processes of all living organisms. It is important for growth, bone development, protein synthesis, gene transcription, cell division and for a strong immune system. Therefore, the United Nations considered zinc as "Life Saving Commodity". Zinc also plays an important role in carbohydrate metabolism because it is incorporated into insulin. Due to the deficiency of it in the human body, it leads to a decrease in insulin, which further cause impaired glucose tolerance [8, 9]. Milk products, meat, nuts and grains are the main sources of zinc for the human body.

It is also essential for birds because it helps in their normal growth, feathering, hormone production, bone development, protein synthesis, nucleic acid synthesis, reproductive performance and for normal metabolic functioning of biochemical enzymes [14].

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Low zinc concentration may cause decreased growth, feed intake, and less production of insulin-like growth factor-I, growth hormone binding protein and growth hormone receptor. Generally, zinc is used in poultry diets in the form of inorganic feed-grade zinc, zinc chloride, zinc oxide, or in the form of organic acid and amino acid chelate. Nanoparticles of zinc oxide are also used in poultry diets as a feed additive. These nanoparticles fulfil all the basic requirements of the body by promoting the growth and feed efficiency. It also improved the levels of total protein, glucose, cholesterol and albumin [5, 12, 19, 24, 25]. There are some zinc compounds such as zinc phosphide that is widely used in insecticides and rodenticides which are highly toxic to birds, fish and some non-targeted mammalian species. Various workers studied that ingestion of metal phosphides may cause hepatic damage and acute liver failure, which leads to biochemical and histopathological alterations [20, 22].

The purpose of this study is to determine the toxic effects of zinc on the liver of broiler chicks.

Methods

Ethical statement

Animal studies were conducted according to the regulations of the Institute Animal Ethics Com mittee (IAEC). Registration no.: 34/1999/CPCSEA, ID no.: CDRI-Tox/SYS/2008/01.

Study design

The experiment was conducted in the Laboratory of Reproductive Biology, D.G. College, Kanpur and Animal House of Central Drug Research Institute (CDRI), Lucknow.

Broiler chicks were quarantined for 10 days and it was confirmed that they were free of pathogen and any other disease.

Broiler chicks were kept in conventional condition (open system) and housed in stainless steel cages (800 × 14 cm^2) in an animal house with room temperature 22 ± 3 °C, relative humidity 50-70%, photo period of 12 h. light and 12 h dark. They were provided with commercial broiler chick starter diet and water ad libitum.

Experimental procedure

Twenty chicks were distributed into four groups randomly with one control group (five chicks) and three treated groups, i.e., low (five chicks), intermediate (five chicks), high (five chicks) dose group in the experiment. The chicks were distributed so as the average body weight of each group remains approximately the same.

This experiment was conducted to determine the toxic effects of Zinc on histopathology of the liver of broiler chicks of various levels of Zinc added to the diet of chicks. Control group was fed on the basal diet (commercial broiler chick starter diet) while all treated groups were supplemented with 300 mg/kgb.w (low dose, LD), 600 mg/kgb.w (intermediate dose, ID), and 900 mg/kgb.w. (high dose, HD) of zinc for 21 days.

Experimental animals

Twenty day-old broiler chicks (*Gallus gallus*) of Caribro breed weight ranging from 35-45 g, were used in the experiment. Broiler chicks were purchased from Gajaria farm, Lucknow.

Chemical used

The experimental animals were fed with zinc, in the form of zinc dust or zinc powder. It is a bluish-grey coloured pure metal powder. It is insoluble in water with boiling point 907 °C and melting point 419 °C.

Sacrifice

After collecting the tissues the animals (20/20) were decapitated by anaesthesia using Ketamine of dose 0.1 ml/20 gm in the morning by 11 AM in the laboratory.

Histopathological studies

Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease. Systemic studies of the compound were analysed by standard histopathological techniques. Liver was taken for histopathological studies and kept in normal saline solution. After weighing, the organ transferred into a formal saline solution for 72 h, for fixation of tissue. Tissue kept in formal saline is taken out and kept in running tap water for 1-2 h. After washing, 4 to 5 mm small slices of tissue were taken and dehydrated with acetone and benzene. After dehydration, tissues were embedded with paraffin wax and blocks were prepared on block making work station SHANDON HISTOCENTRE-2. Surplus wax was removed by trimming. After trimming blocks were kept in ice and 0.5 μ thin microtomical sections were cut and the ribbon was formed by LEICA RM2155 MICROTOME. Float the section on a water bath (temp. 43-47 °C) and removed the wrinkle. Immersed the albumenized slide in the water and brought the sections gently on to the centre of the slide. Kept the slide on a slide warmer (temp. 43-47 °C) till the water was removed.

After section cutting, the sections were stained with haematoxylin and eosin stain. The process of staining is:

I. Put the slides in a slide carrier.
II. Then put those slides in jars containing xylene for 2 changes of 5 min each (Deparaffinization)
III. Transferred the tissue in absolute alcohol for 5 min.
IV. Dehydrated the tissue in descending concentration of ethanol viz 90%, 70%, 50%, 30% each change of 5 min.

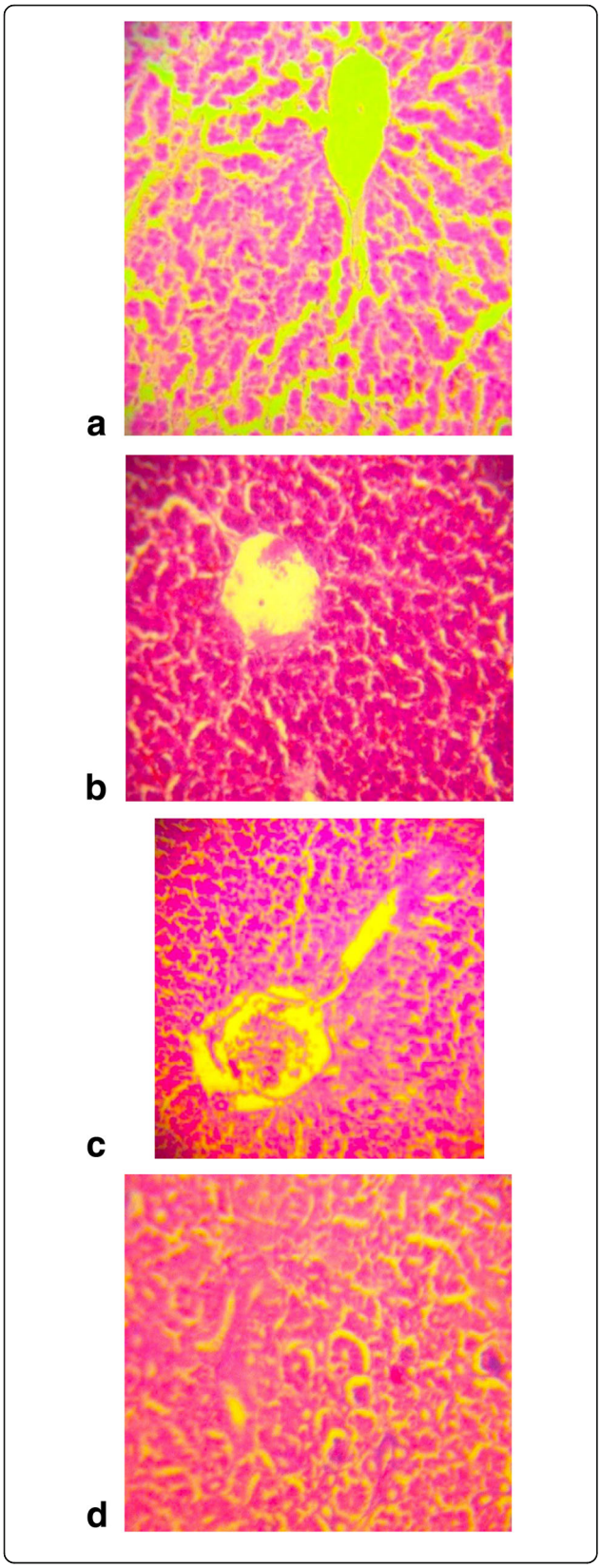

Plate 1 **a** H × E staining of liver tissue from control **g**roup showed normal structure with no evidence of histopathological changes, × 100**. b** H × E staining of liver tissue from zinc (low dose) administered broiler chick showed necrosis and liver cell hypertrophy, × 100. **c** H × E staining of liver tissue from zinc (intermediate dose) administered broiler chicks showed fuzzy liver cells, lymphocytic inflammation and liver cell hypertrophy, × 100. **d** H × E staining of liver tissue from zinc (high dose) administered broiler chicks showed congestion with connective tissue hyperplasia, bile duct proliferation and liver cell hypertrophy, × 100

V. Transferred the slides to a jar containing water.
VI. Transferred the slides in a jar containing haematoxylin for 3-5 min.
VII. Transferred the slide under running water for 5 min.
VIII. Transferred the slide carrier in a jar containing 1% acid alcohol 70% for differentiation 3-4 dips (5 to 30 s).
IX. Washed under running tap water for 5-10 min.
X. Transferred the slide carrier into a jar containing 1% eosin for ½ to 1 min.
XI. Dehydrated the slide in 2 changes of acetone of 1 min at each change.
XII. Transferred the slide carrier to a jar containing 1:1 xylene:acetone mixture for 1 min.
XIII. Then transferred the slide carrier to a jar containing xylene.

After staining the sections are mounted with DPX (Deoxy Plasticisor Xylene) and microscopical examination was done by using a compound microscope.

Results

In this study, the effect of high, intermediate and low concentrations of zinc on the histology of the liver of broiler chicks was investigated. Liver of control birds showed normal structure of central vein, hepatic sinusoids, endothelium, portal vein, sinusoids and kupffer cells, which was influenced by the administration of different doses of zinc (Plate 1a). Following exposure to low dose (300 mg/kg b.w.) (Plate 1b), necrosis and liver cell hypertrophy were found in the liver of birds, but the structure of central vein, hepatic sinusoids, endothelium, portal vein, sinusoids and the kupffer cells as compared to their respective control.

Birds exposed to intermediate dose (600 mg/kg b.w.) (Plate 1c) of zinc it showed the fuzzy liver cells, lymphocytic inflammation and liver cell hypertrophy as compared to control.

Broiler chicks exposed to high dose (900 mg/kg b.w.) of zinc showed pronounced changes in the liver such as congestion of blood vessels, connective tissue hyperplasia, bile duct proliferation, dilation of sinusoids, damaged intercellular contacts between hepatocytes, liver cell hypertrophy and accumulation of inflammatory cells including heterophiles and lymphocytes in the sinusoids of liver as compared to their respective controls (Plate 1d). These results

suggest that due to the more accumulation of zinc in the liver of the broiler chicks, the chicks possess more stress and severe histological changes in their liver because the liver is the important site for zinc accumulation.

Discussion

The liver has the critical job of maintaining the body's metabolic homeostasis. This includes the processing of dietary amino acids, carbohydrates, lipids and vitamins: removal of microbes and toxins in splanchnic blood and route to the systemic circulation; synthesis of many plasma proteins; and detoxification and excretion into the bile of endogenous waste products and pollutants xenobiotics. Zinc is an essential trace element which is necessary for normal functioning of more than 300 enzymes. There are several studies available of oral zinc toxicity which includes various zinc-induced physiological changes in humans and animals, including decreases in the activity of copper metalloenzyme , gastrointestinal effects, haematological effects [7, 11, 17, 21].

Loganathan et al. [16] have been reported in their study that haemorrhage nuclear pyknosis, degeneration of hepatocytes and severe necrosis in the liver of *Labeo rohita* exposed to zinc.

Supplementation of zinc causes cirrhotic liver that leads to a decrease in collagen, fibrin and reticulin reported by Dashti et al. [6]. Ruqayah Ali Salman [18] reported that the deposition of amyloid-like substance on the wall of liver sinusoids, infiltration of inflammatory cells such as neutrophils and mononuclear cells, congested blood vessels and replacement of hepatic cords deposition in albino male mice exposed to zinc oxide nanoparticles for 15 days. Loss of normal liver arrangement with dilated congested veins, inflammatory cellular infiltration, necrotic foci between hepatocytes, dilated congested portal vein with the proliferation of bile duct and blood sinusoids with large cytoplasmic vacuoles in hepatocytes in rat induced with zinc oxide for five constitutive days orally [1]. Li et al. [15] reported higher egg laying in chickens fed dietary supplementation with additional 24, 48, 72, 96 and 120 mg Zn/Kg up to 65 weeks of age. Improvement in the food conversion ratio and significant increase in dietary BMC level and body weight was found in broiler supplemented with photogenic mixture in the diet contained equal ratios of black cumin, *Moringa oleifera* and chicory seeds [2].

Conclusion

Zinc is widely used in the poultry industry for the growth of broiler chicks, but in the limited amount. If we use zinc in higher than normal amount than it affects the liver and other organs of the body of the chicks. Therefore, it is necessary to take a normal dose of zinc, which helps to improve the proper growth of the chicks.

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Authors' contributions
The author(s) read and approved the final manuscript.

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Availability of data and materials
Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Ethics approval and consent to participate
Animal studies were conducted according to the regulations of the Institute Animal Ethics Committee (IAEC). Registration no.: 34/1999/CPCSEA, ID no.: CDRI-Tox/SYS/2008/01.

Consent for publication
Not applicable

Competing interests
The authors declare that they have competing interests.

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