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Hepatic damage associated with fatal zinc phosphide poisoning in broiler chicks

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Abstract Zinc phosphide (Zn_3P_2) is a widely used rodenticide which has the potential to cause high mortality if ingested. The present study was designed in order to explore the hepatic injury in broiler chicks that were acutely intoxicated with Zn_3P_2. For this purpose, a total number of 12 broiler *Saso* chicks were divided into two equal groups. Birds of the first group were exposed to 300 ppm Zn_3P_2 via food. Hepatic damage of intoxicated birds was evaluated biochemically and histologically using the transmission electron microscope and subsequently compared with another healthy non-treated controls (second group). The serum activity of aspartate aminotransferase (AST) was significantly higher in those poisoned with Zn_3P_2, While, activities of both Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), as well as, zinc concentration of hepatic tissue did not represented a significant difference between treated and control birds. Histological examination revealed presence of numerous heterogenic shaped mitochondria in hepatocytes of non-treated birds. Glycogen deposits were also scattered in the form of large electron dense deposits. Kupffer cell was irregular in shape and had numerous pseudopods often projected into sinusoidal lumen. In hepatic cells of intoxicated birds, mitochondrial swelling with cristolysis, few glycogen deposits, vacuoles in the cytoplasm and shrunken darkly stained nuclei are the major ultra-structural changes which were detected. It was concluded that the mitochondria could be one of the main target in hepatocytes for the toxic effect of Zn_3P_2 in broiler chicks.

1. Introduction

Zinc phosphide (Zn_3P_2) is a dull greyish black powder which has been used as rodenticide since the early 1930s [1]. Accidental toxicity by Zn_3P_2 has been reported in non-target animal species including birds [2]. Zn_3P_2 has a disagreeable odor resembling acetylene or rotten fish. It is hydrolyzed in the acidic environment of the stomach, liberating phosphine gas and free radicals [3]. Phosphine acts as a respiratory poison. It blocks the enzyme cytochrome C oxidase as a result of which mitochondrial oxidative phosphorylation is inhibited, causing, in turn, the cells to die rapidly. The inhibition of mitochondrial cytochrome C oxidase leads to pulmonary and cardiac toxicity.

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This can lead to a blockage of mitochondrial electron transport chain [4].

Although lungs, heart, gastrointestinal tract and kidney are considered the major targets for Zn_3P_2 in chickens [5], it is also known that phosphine can induce hepatic dysfunction, especially after the first day of poisoning. The most frequently observed histopathological lesions in the liver were sinusoidal congestion, cytoplasmic vacuolization, centrilobular necrosis, dilated central vein and nuclear fragmentations [6–8]. According to Gupta [9], phosphine causes CNS depression, irritation of the lungs, and damage to the liver, kidney, heart, and CNS. Death occurs as a result of heart failure and/or pulmonary edema. Following a large dose, death usually occurs within an hour, while with smaller doses, death can occur between 4 and 72 h.

However, there are insufficient data about the ultrastructural changes of chicken hepatocytes after the exposure to lethal dose of Zn_3P_2.

As described in the previous studies carried out by Purton, Ohata and Ito [10–12] the ultrastructure of chicken liver parenchyma was fundamentally similar to that of mammalian liver; however, there were a few differences. The inter anastomosing hepatocyte plates as recognized and termed in mammalian liver were more like cords or tubules in the chicken. They were two cells thick, limiting the contact between surface of hepatocytes and blood to only one side of the cell. In the past this has given rise to the suggestion that the mammalian liver structure as sheets of single cell thickness provided a better constructional stability compared to that of the avian species. Also, it is essential for the greater physiological efficiency by increasing the contact surface between hepatocytes and blood [13].

In this study, the ultrastructural modification in chick's hepatocytes after acute experimental Zn_3P_2 intoxication as well as the associated serum biochemical alterations with special attention to hepatic zinc concentration as a possible marker element in cases of suspected Zn_3P_2 poisoning are scrutinized.

2. Materials and methods

All procedures conducted in this experiment were approved by the local authorities (Faculty of Veterinary Medicine, Alexandria University, Egypt).

2.1. Birds and experimental design

Twelve broiler *Saso* chicks were obtained from a commercial hatchery on their first day of life and were housed in cages. They were provided by incandescent light for heating (30 ± 2 °C) and with free access to food and water. Birds were kept under supervision for 2 weeks without any medication for detection of any abnormal signs or behavior, and then were divided into two groups of 6 birds in each. The chicks of the first group were exposed to a commercial diet (yellow corn, soyeabean meal, gluten, minerals and vitamins with 23% protein) supplemented with commercial product of Zn_3P_2 (RAT KILL 80%) at dose of 300 ppm, while the birds of the second group served as control and not subjected to any type of treatment. Chicks were clinically observed for signs of toxicity and a particular attention was paid to time of death.

2.2. Sampling

At the moment of death, which guided by gasping (stop of respiration), blood samples were collected from wing vein in sterile test tubes without anticoagulant to obtain serum for liver functions assay. In control birds, blood was collected from wing vein and then euthanized by slaughtering. Quickly after death, liver was rapidly extracted from birds and divided into two parts: The first part was kept frozen at −20 °C until the assessment of zinc concentration. The second part was rapidly submersed in cold 4F1G fluid which consists of 4% formaldehyde and 1% glutaraldehyde for transmission electron microscopic examination.

2.3. Liver function assay

Serum activity of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were measured spectrophotometrically using commercial diagnostic kits obtained from Vitro Scient Co. (Egypt) according to the manufacture instructions.

2.4. Zinc concentration

Zinc concentration in liver samples was measured using 4100 MPAES Spectrometer (Microwave Plasma-Atomic emission Spectrometry: Agilent Tech., Santa Clara, CA, USA) at wave length 213.857 nm according to the method described by Jorhem and Engman [14]. Metal concentration is expressed on a wet weight basis as ppm.

2.5. Examination of hepatic tissue using transmission electron microscope

Pieces of 1 mm were cut from the liver samples using sharp blade and quickly fixed in 6% solution of phosphate buffered gluteraldehyde pH 7.4 for 6 h at 4 °C [15]. After initial fixation, the tissues were washed several times in cold (4 °C) 0.1 M phosphate buffer every 15 min for 2 h. The tissues were post fixed in 1% solution of cold osmium tetroxide (OsO_4) 0.1 M buffer pH 7.2 for 2 h. Then, rapidly dehydrated through ascending grades of ethyl alcohol and transferred to a 1:1 mixture of propylene oxide and epoxy araldite. Semi- thin sections (1 μm) were cut firstly and stained with toludine blue and viewed with light microscope to select the suitable areas for the electron microscope examination. The ultrathin sections (60–100 nm) were cut by a glass knife with LKB microtome, and stained with uranyle acetate followed by lead citrate [16]. These sections were examined under the transmission electron microscope (JEOL 100cx, USA) operating at 80 kV.

2.6. Statistical analysis

Data were expressed as mean ± standard error. The significance of the difference between treated and control parameters